Influence of Intraspecific Relationships on the Allocation of Temporal Resources in the Lekking Ruff, *Calidris pugnax*

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Abstract

Alternative Reproductive Tactics (ARTs) are utilized by animals of both sex to optimize their reproductive success in an intrasexually competitive environment where they are unable to prevail through use of the predominant tactic for obtaining mates. Often manifested as subtle differences in physical size, species where distinct genetically fixed morphological differences are associated with specific tactics are rare. The ruff (*Calidris pugnax*), a lekking palearctic wader, exhibits three fixed morphs (territorial and combative "independents", cooperative and non-aggressive "satellites" and the female mimicking "faeders") with distinct morphologies and reproductive behaviours which have their reproductive success interwoven to one-another on the leks. This diversity creates intricate connections between the morphs and sexes, giving rise to a web of influential relationships that have not been investigated thoroughly. These relationships were explored through utilization of stand-alone cameras to obtain precise information on the time use and reproductive success of male morphs and females and the evolution of their time investment throughout the lekking season. The degree of influence morphs and sexes exerted on the allocation of temporal resources by others through their own corresponding time investment was particularly investigated. The gradual progress of the female receptivity eventually resulting in a rapid copulatory peak was described, as well as and the central role of the time investment by dominant males on the attractiveness of a lek, both to females and other males alike. Indications of a more complex role of the satellite males in the overall success of a lek were identified, suggesting of even greater intricacy in the connections between the morphs.

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1. Introduction

1.1 Alternative Reproductive Tactics

Alternative Reproductive Tactics, referred to as ARTs, are consistent intraspecific and intrasexual variations in reproductive behaviours. They serve the purpose of maximizing the number of fertilizations obtained by an individual animal, both male and female. A *strategy* is defined as a set of decision rules with a genetic basis that give rise to *tactics***,** which are behavioural phenotypes resulting from a strategy. Multiple different tactics can be included within a strategy (Gross 1996; Taborsky *et al*. 2008).

ARTs are generally characterized by traits that have evolved towards the same functional end and display discontinuous distribution. This can be observed in many forms, such as size dimorphism, colour polymorphism, dimorphic morphological structures and behavioural alternatives. There are multiple pathways for ARTs to manifest in an individual (Figure 1). A genetically fixed tactic occurs inevitably but if fixation is developmental and alternatives exist, environmental conditions may influence which tactic is manifested. Variation in tactics utilized by monomorphic individuals can rise from multiple factors, such as resource availability, level of competition or passing a threshold such as size or age (Taborsky *et al.* 2008).

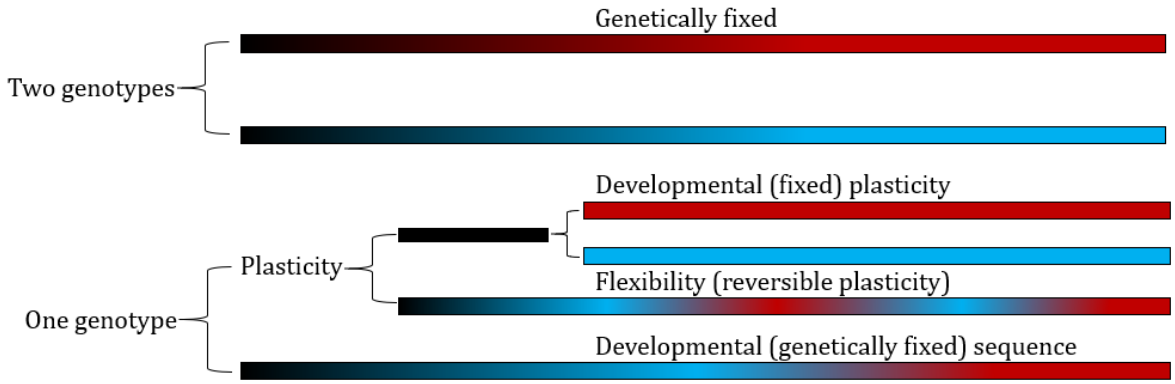


Figure 1. A simplified overview of possible developmental Alternative Reproductive Tactic pathways. Red and blue colours represent different tactics whereas black colour represents an immature state where no tactic has manifested. Edited from Shcradin (2019).

In a classical paper on Alternative Reproductive Tactics, Gross (1996) introduced three categories of strategies (Figure 2):

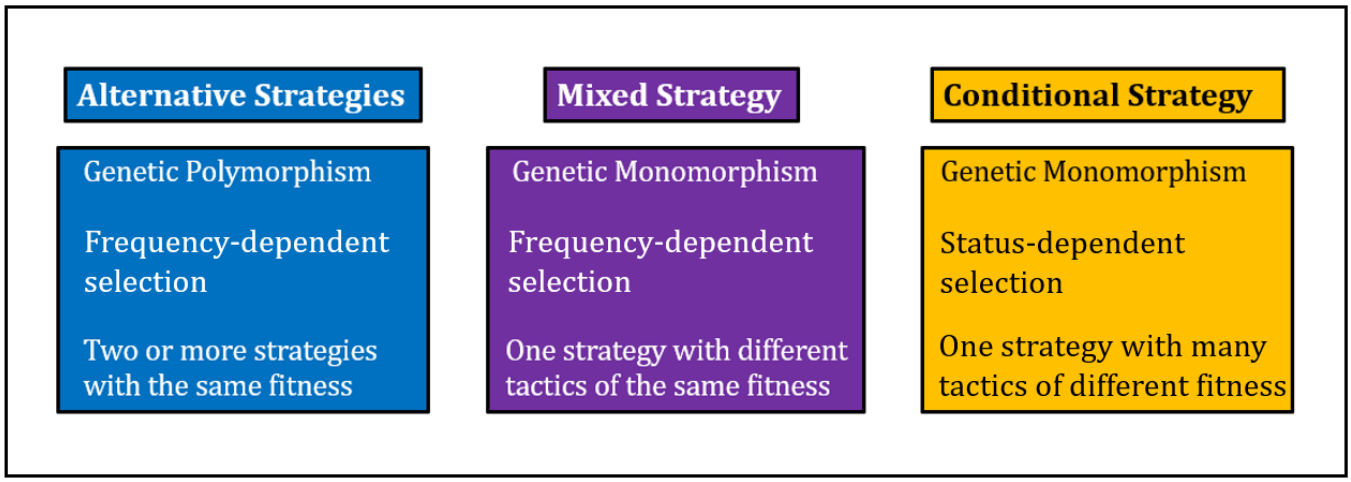


Figure 2. Categories of reproductive strategies by Gross (1996), edited from Schradin (2019).

Alternative strategies are genetically fixed, polymorphic and based on frequency-dependent selection where the prevalence of a phenotype in a population determines its fitness level. This can manifest as positive frequency-dependence, where increased frequency of a phenotype increases its fitness, or as negative frequency-dependence where commonness decreases a phenotypes fitness. Application of different tactics result in the same average fitness in certain equilibrium frequencies.

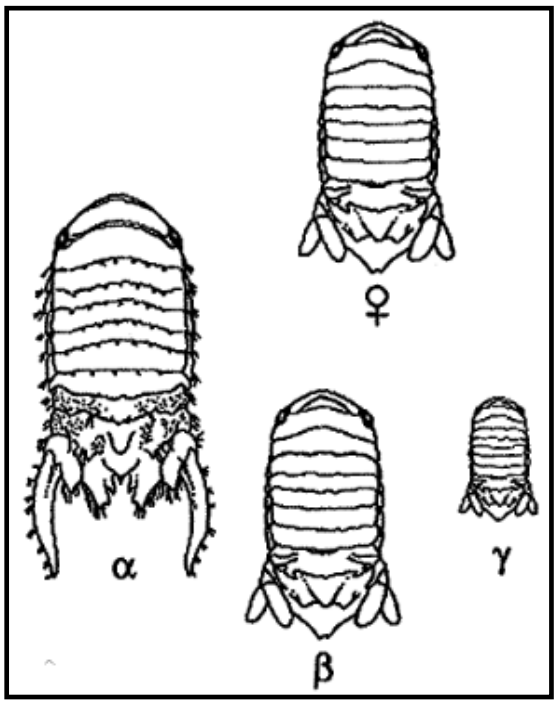


Figure 3. Morphs of *Paracerceis scultpa*: α, β, and γ - males and a female. Edited from Bekoff 2004.

An exemplary case can be found in *Paracerceis sculpta*, a marine isopod, where *α, β,* and *γ*-male morphs (Figure 3) are determined by three alleles at a single autosomal locus.

This major *Ams* (*Alternative mating strategy*) gene is of Mendelian inheritance and the alleles at the locus exhibit directional dominance of $Ams^{\beta} > Ams^{\gamma} > Ams^{\alpha}$. The large fighter α-males collect harems of females within sponges, which they defend from intruding males. The female-imitating β-males hide and mate within the harem, whereas the minuscule γ-males resemble larvae and rapidly sneak to mate with the females.

The α -males mature in 6 months and use 20% of their body for sperm production. The β -males mature faster and use 50% of their body for sperm production whereas the γ -males mature very rapidly and devote over 80% of their body to sperm production. The relative fertilization success of the morphs varies depending on factors such as density of receptive females and frequency of other male morphs. In a spongocoel with a single female, the α-male sires most of the offspring, but in harems of multiple females the fertilization rate of a β-male rises to 60% whereas the success of a γ -male increases linearly with harem size. The average fitnesses of the three morphs, however, are equivalent (Shuster & Wade 2003; Shuster & Arnold 2007).

Mixed strategies with alternative tactics are described by Gross (1996) as theoretical possibilities of individuals each expressing an appropriate mix of tactics as alternatives. They are genetically monomorphic, based on frequency-dependent selection and characterized by a probabilistic basis: The probability to utilize tactic A is "x" whereas the probability to use tactic B is "1-x". Developmentally fixed plastic tactics, where the average fitness is equal, and choice of tactic depends on the frequency of all tactics utilized by a population. The number of possible influential environmental factors would make the existence of two individuals with the same status highly unlikely. Empirical evidence of mixed strategies remain weak and absent from the literature (Schradin 2019).

Conditional strategies with alternative tactics are genetically monomorphic and based on status-dependent selection. In this model of selection utilization of a tactic depends on the status of an individual in comparison to its rivals. As the fitness yield of tactics are different, individuals should apply the most beneficial one after passing a certain threshold, such as age or degree of physical capability, and resort to a "best of a bad job" tactic with lower fitness results, which still triumph over no reproductive success whatsoever, when below it or faced with superior competitors (Taborsky *et al.* 2008).
In the most basic form two alternative strategies are utilized by males: the bourgeois and the sneaker/satellite. Bourgeois males are often large, dominant and follow the tactic with the greatest fitness payoff. The sneaker/satellite males have lower competitive capabilities, due to age or lesser essential armaments. A "satellite" strategy can be especially effective in species with external fertilization. Horseshoe crab (*Limulus polyphemus)* males mount and attach themselves to females heading for intertidal spawning beaches. On arrival they are joined by satellite males, which are worn and in poorer condition than the attached males (Brockmann & Penn 1992). These satellites remain in the vicinity of the couple and partake in sperm competition when the eggs are laid. Though paternity analysis Brockmann *et al.* (1994) found that on average the satellite males fertilized 40% of the eggs.

African striped mouse (*Rhabdomys pumilio)* males display three phenotypically plastic ARTs: 1.) Heavyweight territorial breeders living in groups. 2.) Lightweight, natally philopatric group dwellers that sneak copulations from neighbouring female groups and 3.) Intermediate sized solitary roamers, that breed with all females (Schradin & Lindholm (2011). Tactics readily change as group demographics shift and males gain body mass, their entire reproductive investment is determined by their chosen tactic for the single breeding season they live through (Schradin *et al.* 2010).

Overlap in the use of the terms *strategy* and *tactic* exists in the literature, as does some confusion between the definitions of types of strategies. The main difference between mixed and conditional strategies comes in the form of different predictions for fitness consequences of different tactics, equal for mixed and variable for conditional, and the outcome is mainly influenced by environmental factors rather than noticeable differences in the strategies themselves. To clarify the terminology, Schradin *et al.* (2010) proposed for both mixed and conditional strategies to be coined under the category of *Single strategy*. Genetically monomorphic single strategies are influenced by both frequency- and status-dependent selection and one strategy gives raise to multiple tactics. All individuals follow a highly similar set of decision rules, independent of current fitness consequences of ARTs, and the success of tactics determined by the environment they are applied in.

In majority of studied cases the variation between individuals utilizing differing tactics is mainly behavioural along with an often-unobvious difference in body mass. Cases where ARTs are associated with distinct morphological differences are far rarer (Schradin 2019). Species that display ARTs do not only provide a platform for studying complex behaviours and their development, but also evolution and ecology of extraordinary physiological features and their function (Lozano *et al.* 2013). The underlying processes behind the divergence of fixed phenotypical characteristics associated with reproductive tactics have proven to be a source of remarkable evolutionary innovations. Numerous unanswered questions exist regarding the success of tactics and morphs that at first glance appear to be at a disadvantage but must possess higher fitness than the common ones. The co-existence of multiple tactics at an equilibrium comes across unlikely, and furthermore the existence of complex relationships between such individuals

One extensively studied species, in which highly distinctive phenotypical characteristics are associated with alternative reproductive tactics and intricate intrasexual relations, is the ruff (*Calidris pugnax*) (Hogan-Warburg 1966; Van Rhijn 1973; Lank & Smith 1987; Lank *et al.* 1995; Lozano *et al.* 2013; Küpper *et al* 2016).

2.0 Ruff: A Life history

2.1 Description

The ruff is perhaps the most distinct of all wading birds in the order Charadriiformes, possessing a unique collection of features. The ruff displays a remarkable degree of sexual dimorphism but unlike in most of the species of the Scolopacidae family, it is the male that is the larger sex. Average male body mass ranges between 170-200g, and it is the largest calidrine sandpiper, whereas the females, also known as "reeves", fall between 100-130g and the size difference in wing length of males (172-198 mm) and females (136-167 mm) is clear (Pearson *et al.* 1970; Pearson 1981; Van Rhijn 1985; Jehl & Murray 1986; Höglund & Lundberg 1989).
The most striking feature is the male nuptial plumage (Figure 4) which consists of a ruff of elongated neck-feathers, two tufts on the crown of their head and a collection of facial warts surrounding the eyes and the base of the bill (Höglund & Lundberg 1989). The coloration and patterns are hypervariable and range from white to black, blue to red and can come with irregular patterns or regular spots, bars and stripes. The specific characteristics are retained through life, making identification on an individual level possible and relatively easy (Andersen 1948; Hogan-Warburg 1966). The plumage is intrinsically affiliated with three genetically fixed male morphs (Section 2.3) that utilize different reproductive strategies. The most common male morph is the aggressively territorial, diversely coloured *independent*. The non-territorial *satellite* males are predominantly white whereas the rare *faeder* male closely resembles a female (Jukema & Piersma 2006).
The females show a far lesser degree of nuptial plumage that only manifests as somewhat brighter colours and more conspicuous patterns in the feathers of the head and upper body, with individual variation usually being too vague to allow identification of individual reeves (Hogan-Warburg 1966).

Figure 4. Male ruffs in full nuptial plumage at a traditional lek site (Photo: Kari Koivula).

2.2 Range & Habitat

The ruff is a palearctic sandpiper that breeds in northern Europe and across Siberia (Figure 5), occupying a variety of inland wetland habitats, ranging from floodlands to marshes and river deltas (Küpper 2016). The birds arrive in their breeding grounds in late April-early May. The males leave in late June-July with females and juveniles departing in late July-early September. There is a marked difference in the distribution of the sexes in the wintering grounds (Gill *et al.* 1995). Males show a tendency of remaining primarily in Europe and North Africa, whereas the females congregate in West and South Africa, where they outnumber the males in winter flocks by 10-15 to 1. Remaining in proximity to the southern edges of the breeding range likely allows males to occupy prime breeding grounds more rapidly than their further migrating rivals (Morel & Roux 1973; Blotzheim *et al.* 1975; Schmitt & Whitehouse 1976). An increasingly eastward redistribution of the European ruffs has been identified to be connected to decline of the traditional staging grounds in the Netherlands due to modern agricultural management practices (Verkuil *et al.* 2012). Some populations migrate to south-east Asia and even Australia (Küpper *et al*. 2016).

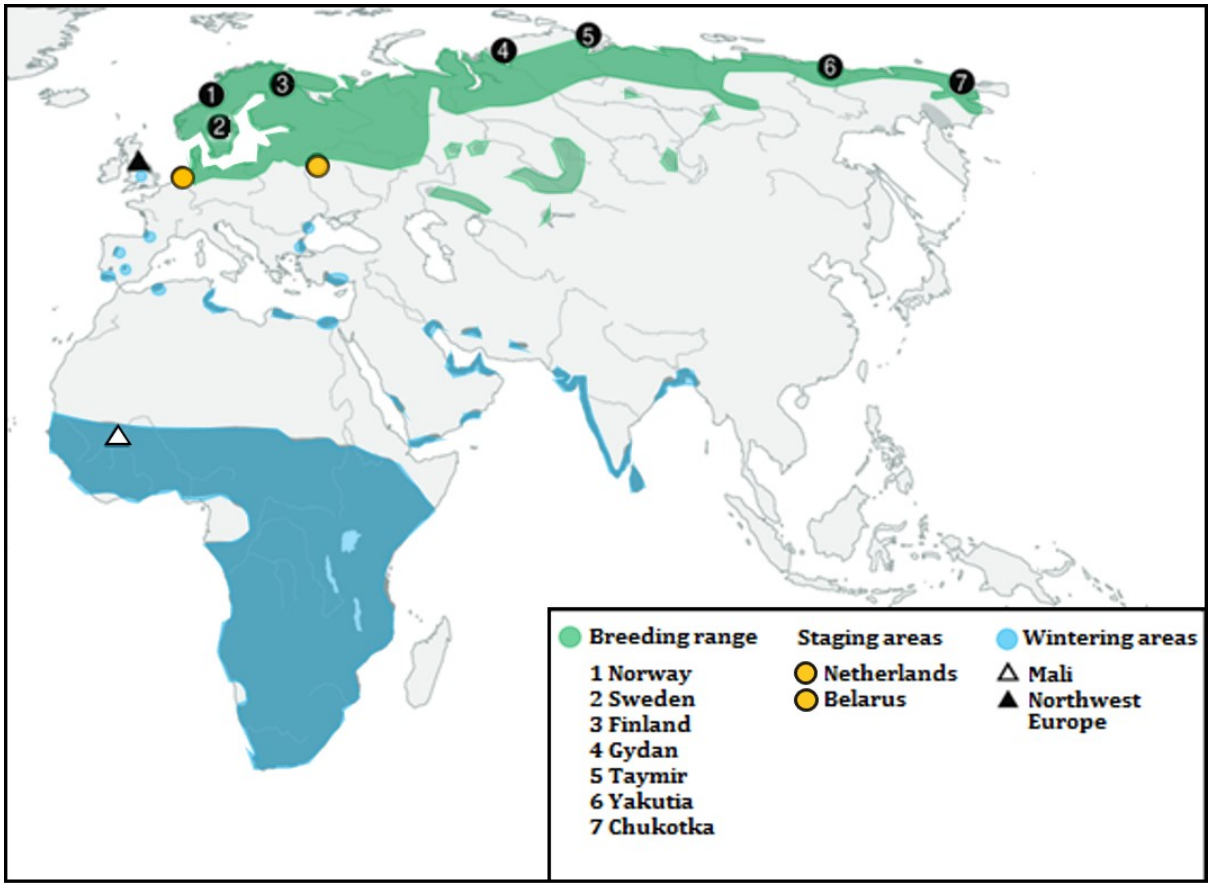


Figure 5. Main global distribution of the ruff (Breeding range in green, wintering areas in blue, notable staging grounds marked by yellow circles) edited from Verkuil (2010).

The European population has diminished during the last three decades**,** with over 85% reduction that has been especially severe in wet grassland habitats. The breeding range used to extend as far south as Hungary but has continuously shifted northward during the last century. Today the southernmost nesting areas are in the Netherlands and northern parts of Germany and Poland. Nonetheless it is globally classified as a species of least concern (IUCN 2019) as the ruff remains one of the most common and most widely distributed wading bird species in Eurasia, with breeding population estimates ranging between 2,3-2,8 million birds out of which 95% breed in the Russian tundra (Zöckler 2002; Küpper *et al.* 2016).

The Finnish population has followed the European trend and has been found to have reduced by over 50% between 1974 and 2010 (Valkama *et al.* 2011). Formerly widespread, it has become a rare breeding species outside of Lapland and the eastern coast of the Bay of Bothnia**.** The current number of breeding pairs is estimated to be between 5,000 – 8,000 and it has been declared an endangered species. The falling numbers may be due to anthropogenic interference of the wintering grounds in western Africa, environmental pollution in both wintering and breeding grounds and climatic factors driving the population to a more north-eastern focus (Zöckler 2002; Grishanov 2006). The high degree of sensitivity to changing water tables,

eutrophication and climate change would suggest that the ruff could be an ideal indicator-species for northern wetland environments.
The ruff is largely insectivorous throughout the summer, with especially the young relying on terrestrial and aquatic insects and their larvae. The diet is complemented with plant matter and seeds, especially rice, during the winter (Trolliet & Girard 2001; Küpper 2016).

2.3. Male morph variation and its genetic background

The existence of two distinct male mating strategies and their association to certain morphology is apparent in the first published observations of interactions between males on leks by Selous (1906-1907). The behavioural split has been referred to as "behavioural polymorphism" (Hogan-Warburg 1966), "behavioural dimorphism" (Van Rhijn 1973) and "diethism" (Shepard 1975). Studies on the relationships, life history effects and evolutionary causes of these strategies have been intensively studied for decades. Behavioural observations of both males and females have been conducted in the field across Fennoscandia and in the Netherlands, where Jukema & Piersma (2004; 2006) first described the third male morph, the faeder.
Both Hogan-Warburg (1966) and Van Rhijn (1973) deemed it probable that the differences between independent and satellite males rose from genetics, but lack of possibilities to conduct breeding experiments prevented conclusive answers at the time. A captive population of birds was established from eggs collected in Finland between 1985-1990 first in Kingston, Ontario and later at the Simon Fraser University, Burnaby, in 1994 (Lank *et al.* 1995) to facilitate controlled pedigree breeding and genetic studies. Two faeders were added to the population in 2006. Meticulous gathering of information on the inheritability of traits and behaviour of the captive birds and eventually through evolution of genetic methodologies allowed Küpper *et al.* (2016) to uncover that the genetic processes determining male morphs were governed by a 4,5 Mb supergene, containing 125 genes, located on avian chromosome 11.
The inversion event that led to the divergence of faeder and independent alleles has been estimated to have occurred 4.09 ± 0.16 million years ago and the divergence of satellite and independent alleles 3.87 ± 0.15 million years ago. The satellite chromosome appears to have risen through a recombination event between independent and faeder-like chromosomes 520,000 ± 20,000 years ago, resembling the generation of a third allele at the *Rose-comb* locus in chicken which alters their cranial comb development (Imsland *et al.* 2012; Lamichhaney *et al.* 2015).
The inversion and the subsequent recombination have altered the physiology of the carrier morphs on multiple levels. Changes in steroid metabolism underlie the distinct external features and behavioural patterns seen in satellites and faeders. High levels of circulating testosterone

have been documented in the independent males, whereas in satellites and faeders the concentrations of androstenedione have been found to be higher (Küpper *et al.* 2016). Evidence of independents displaying a stronger cell-mediated immune response in the breeding season, likely related to their higher risk of injury during lekking, was found by Lozano & Lank (2004). Yet unexpectedly male ruffs appear to exhibit stronger immune responses during the nonbreeding season (Lozano *et al.* 2013) which may indicate of a trade-off between allocation of resources to reproductive prowess over immune defences, or perhaps of higher pathogen risks in their wintering grounds.

2.3.1 The Independent Male

Figure 6. Independent male ruff with a female in the background (Photo: Kari Koivula).

The Independent males display predominantly dark nuptial plumage which can range from jet black to bright yellow with an extensive array of variations in patterns (Figure 4, Fig. 6, Fig. 8). Independents are heavier with longer wings, tarsus and overall body size than the other male morphs (Höglund & Lundberg 1989). Independents can be further categorized into two behavioural classes, *residents* and *marginals*, depending on their status on a lek. Male status is usually connected to age and physical condition, with males reaching fully mature nuptial plumage after 3 years of age (Hogan-Warburg 1966). This status is interchangeable, with marginals capable of establishing themselves as residents and residents losing their status and becoming marginals. Residents establish a displaying territory which they fiercely defend throughout the breeding season, forming the core of a lek site. At the height of the breeding season the residents hardly depart from the lek, only occasionally embarking to hastily feed (Howard 1964). Males, especially residents, fatten up considerably before spring migration, facilitating their commitment to the lek (Pearson *et al.* 1970). Residents attentively monitor each other and swiftly return to their display territories from feeding if any action towards approaching reeves or other males is undertaken on the lek. The number of attending residents can vary greatly depending on sites, progress of the season and even by day, but average between 3 and 8 (Hogan-Warburg 1966). Residents rarely visit other leks and if they do, their status will drop to a marginal. Marginal males pay irregular, short visits to multiple leks, often following satellites or females. They are frequently greeted with hostility on arrival from the resident males and usually confined to the margins of a lek. Marginals may develop a preference for a given lek and through numerous visits can get the residents accustomed to its presence. A marginal may eventually establish a permanent position on a lek through perseverance and combat, thus rising to resident status (Hogan-Warburg 1966).

2.3.2 The Satellite Male

Figure 7. Satellite male with chestnut markings on the head (Photo: Kari Koivula).

Predominantly light coloured and sometimes pure white nuptial plumage with various hues or markings (Figure 7) typically distinguishes the *satellite males* from the physically somewhat larger independents (Figure 8). The underlying cause behind the prominence of light coloration in satellites has been proposed to be the location of the MC1R gene within the inverted region of the supergene, in conjunction with altered metabolism of sex hormones. Overexpression of a variant form of MC1R in the ornamental breeding plumage in particular, would explain why satellites and independents are nigh indistinguishable outside the breeding season Lamichhaney *et al.* (2015). The dominance of white has been observed to increase with age (D Lank 2019 personal communication April 23). Although overlap exists with remarkably white independents or satellites with notably colourful plumages, the behavioural differences of the morphs are prominent.

Satellites do not engage in aggressive behaviour nor establish display territories. They form a temporary alliance with a resident male and perform a joint display on their companion's territory. Satellites show opportunistic tendencies and can cooperate with multiple residents but often prefer single, high ranking individual. Multiple satellites can team up with a single resident. The alliance between an independent and a satellite is intricate, and the degree of tolerance towards a satellite depends on multiple factors. On larger leks a higher degree of aggression is directed towards satellites, whereas on smaller leks they are more tolerated. Hogan-Warburg (1966) described two behavioural types of satellite males, the *central* and *peripheral*, with much more subtle differences than resident and marginal independents. Peripherals remain at the margins of the lek in a similar fashion to marginals, whereas the central satellites visit leks more frequently and are allowed to settle in central, often most contested, displaying territories. Satellites often accompany roaming females and visit multiple leks, where their presence is determined by female attendance and independent tolerance. The proportion of satellites has been estimated at 15% of all males (Küpper *et al.* 2016). Although their behaviour and tactics on the leks are well documented, the precise method and time when they acquire the copulations necessary to maintain the morph in the population remains largely unknown (D Lank 2019 personal communication 23 April).

Figure 8. An independent (left) and a satellite (right) male ruff during a lull on a lek (Photo: Kari Koivula).

2.3.3 The Faeder Male

Figure 9. The faeder male is physically nigh indistinguishable from the female (Photo: Vroni Rohr).

The existence of the third male morph was first described by Jukema & Piersma (2004, 2006) and has been found to comprise 1 % of male ruffs (Verkuil *et al.* 2008; Küpper *et al.* 2016).

The *faeder* (old English for "father") males do not exhibit nuptial plumage and are superficially identical to females (Figure 9, Fig. 10) They can be distinguished by wing length and weight, which fall in-between females and independent and satellite males. Faeders utilize a sneaker strategy (Taborsky 1997), attempting to access females when the dominant males are preoccupied or by rapidly mounting females that have solicited to an ornamented male. The faeders have been observed to engage in a multitude of disruptive behaviours such as imitating soliciting reeves and intercepting mountings, to distract and prevent other males from mating with females (D Lank 2019, personal communication, 23 April).

Through selective breeding of captive birds, Lank *et al.* (2013) introduced the faeder allele to a population previously devoid of it. This produced normal-sized ruffs of both sexes, faeder males and unusually small females. Where no visible manifestations of the satellite allele have been detected in the reeves, these diminished females were identified to be carriers of the faeder

allele. Although reduced size is essential for the male faeder breeding strategy, this grants no discernible benefit to the female and may in fact be detrimental to their reproductive success. This results in *intralocus conflict* where selection on a trait in the other sex displaces the other from its optimal phenotype (Bonduriansky & Chenoweth 2009). In their breeding experiments Lank *et al.* (2013) also found that females possessing the faeder allele had not produced any offspring by their third year, indicating that the allele also hindered the reproductive maturation of female carriers. This would indicate that, unless other fitness components counterbalance this disadvantage, the faeder males possess fitness advantages over the other males that on average compensate for this drawback.
The degree to which the other male morphs and females recognize faeders as males is not completely understood, although there have been observations of females soliciting themselves to faeders in captivity (Lozano *et al.* 2013).

2.3.4 The Female

Figure 10. A colour-banded reeve (Photo: Vroni Rohr).

The reeves carry the same genetic morphs as the males, but in their case known physical manifestations are restricted to the small body size of faeder females (Lank *et al.* 2013; Lamichhaney *et al.* 2015). Pedigree studies have approximated the inversion frequencies in females to be about 12% of the population (D Lank 2019 personal communication April 23). The presence and function of the supergene in females was investigated by Lank *et al.* (1999) via a testosterone releasing implant. Reeves with implants rapidly began displaying both independent and satellite male courtship behaviour, grew plumages resembling those of males and increased in weight similarly to males preparing for lekking.
The more subtle behaviour of female ruffs is less studied than that of males, although their interactions with the males and mate-choice patterns have been closely monitored.
Females visit leks alone or in flocks, often followed by marginal and satellite males. Especially early in the mating season the duration of a female visit can range from a few seconds to several minutes, during which the females stand amidst the males, preen themselves and then leave without interaction with the lekking males. Details and extent of female movements and lek monitoring are not well understood.
Receptivity appears to build up over time until copulation occurs quickly at the display territory of a chosen male. This can be followed with another copulation a few minutes afterwards.

Unlike groups such as the Anseriformes where forced copulation is common (e.g. Titman & Seymour 1981), ruff females are the sole deciders of mating partner and time, although the degree to which females willingly consent to intercept matings by faeders is unknown. The degree of female fidelity to a male it has copulated on a previous visit has proven challenging to investigate due to difficulties in identifying individual females. Hogan-Warburg's (1966) observations of few males performing most matings on large leks suggest that females would pick a preferred male and return multiple times. Mildenberger (1953) reported that individually recognizable females were either male exclusive or mated with multiple partners.

Female mate choice has been the topic of multiple studies. Hogan-Warburg (1966) highlighted the size and condition of the male nuptial plumage and that males in poor condition were completely unsuccessful. Central location of display territories and high rate of displays by a were considered to be the key factors of successful male ruffs by Halliday (1983). To resolve conflicting results and previous studies use of simple or no statistical analyses, Hill (1991) utilized multivariate analysis on his observations of two leks on two seasons. The results disputed the relevance of display territory location, display rates and influence of plumage coloration or patterns. Consistent lek attendance was shown to influence the mating success of a male significantly, indicating that the females remember precisely which males were present at a given lek during their visits. Low rates of aggressive behaviour was found to positively affect their success rate, which would be consistent with observations that the most established males have on average the least aggressive encounters and therefore also avoid evicting females as a side effect of intrasexual aggression. Female preference of larger leks was shown to significantly increase the rate of female visits per capita for males attending these leks by Lank & Smith (1992). Although conflicting results regarding importance of factors have been presented, the positive impact of the presence of a satellite male on a display territory has been consistent across authors. The process by which the females assess the independent-satellite union remains unclear. Similarly, the question remains if the females are assessing satellite visits and their duration or are the satellites assessing female visits and acceding accordingly to the most potential lek (Lank & Smith 1987). Yet another unclear aspect is the choice between an independent and a satellite on a joint display. Especially on larger leks the tolerance towards satellites is much lower so their mating success drops as well, resulting in a few independents mating with multiple females in succession. On smaller leks the preference of double-occupied display territories prevails and the dominant independent and accompanying satellite both tend to copulate multiple times (Hogan-Warburg 1966).

3. Reproduction

A cornerstone discovery by Küpper *et al.* (2016) was the lethal consequences of homozygosity of the inversed supergene. The inversion disrupts the function of the centromere protein N encoding *CENP-N* gene, which has an indispensable function in mitosis. This creates a significant handicap to the inversion carrying morphs and also prevents recombination between the two, making a faeder + satellite genotype unviable. To maintain allelic frequencies and the polymorphism, alternative alleles must have equal long-term fitness that exceeds the independents (Sinervo & Lively 1996; Shuster & Wade 2003). This brings forth an extraordinary aspect to animal reproduction and raises a multitude of questions regarding how the satellite and faeder morphs are maintained in the populations.

3.1 Lekking paradox

Leks are gatherings of animals for group displays to attract mates. In lekking species copulations are monopolized by a small number of males. This condition is maintained by extreme selectiveness for specific features or behaviours by females, for which the only resource attained from the males is sperm. Theoretically this should diminish genetic variation of the species and abolish the benefits of choosiness through constantly increasing prevalence of the selected traits, a process known as *Fisherian runaway*. Yet no runaway selection has been detected in lekking species, giving rise to the "*lekking paradox"* of why and how variation in male fitness continues to exist in non-resource based matings systems (Borgia 1979; Reynolds 1990; Kirkpatrick 1991; Miller *et al.* 2007). Proposed resolutions to the paradox have included such as that a courtship display conveying a potentially negative effect on males allows comparative evaluation of their quality by females (Zahavi 1975) and that increasing lek size would increase the average male fitness, prompting aggregations (Höglund *et al.* 1993).

3.2 Lekking in Scolopacidae

Lekking is most prevalent in avians, especially in many species of grouse in the subfamily Tetraoninae. Amongst Scolopacidae, the sandpipers, only three species engage in lekking behaviour. The buff-breasted sandpiper (*Calidris subruficollis*) is a North American species where males occupy large, 200m2 – 1ha territories where they perform both ground-based and aerial displays by flashing their bright white wings (Lanctot *et al.* 1997). The males of the great snipe (*Gallinago media*) of north Eurasia perform their displays on small, 100m2 territories by bursts of clicks accompanied by tail feather spreading and occasional male-on-male combat (Höglund & Lundberg 1987).

A ruff lek is typically situated on an open, grassy area. Birds return to a traditional main lek site annually and form smaller side-leks in the proximity of the main site. The most favoured leks form around suitable nesting habitat (Widemo 1997). Independent males establish approximately 30 cm diameter display territories, trampled, bare of grass and marked by faeces, 1 – 1,5m apart from each other within a main arena. Residents occupy their positions on a lek from before sunrise, followed by an inconsistent flow of marginals. Satellite males arrive alone or accompanying groups of females that begin visiting leks after dawn. The independent-satellite union can form early in the day and last for hours or be formed rapidly on arrival of a satellite trailing females. As reeves depart, a portion of present marginals and satellites often follow in pursuit.

For most of the day residents remain alone and show little activity. If a male departs, it will quickly assume its display territory upon returning. Short bursts of aggression occasionally interrupt periods of inactivity, stimulated by a male moving across the "no-man's land" between territories. Residents strike at each other by pecking, kicking and grabbing. These combat bouts are over in seconds, after which the males return to their territories. Males that have been absent from the lek face similar agonistic acts as they land, as will any marginal male attempting to enter the lek. Marginals are kept at the fringes of the lek by residents and rarely return the aggression shown towards them. Marginals are hostile towards newly arrived contemporaries and often take on opportunities to strike at marginals that have been driven off by residents. Satellite males generally move in the lek without being harassed, although on larger leks aggression towards them is more common. Satellites interact primarily with residents, generally ignoring marginals and other satellites. Two satellites can team up with the same resident but tend to show little attention towards each other (Hogan-Warburg 1966).

A ruff lek is silent, underlining the role of visual structures and displays. The hypervariable plumage of the males may play a role in reducing energy loss and risk of injury of male-male combat due to ease of individual recognition, thus requiring a single encounter to sort out their hierarchy. These precautions may be the underlying reason to their high degree of site fidelity, especially in dominant individuals (Widemo 1997). Severe, extended fights are rare but can lead to the loser losing status and being reduced to a marginal or even being expelled from the lek altogether.

Female arrival or pass-by stir action. Wing-flapping, jumping, posturing and air-pecking acts are combined into a fast-paced performance to entice the reeves. When a female lands on the lek, males remain frozen in a squat at their display territories. Female visits are brief, between few seconds and minutes. During visits they can simply remain in the midst of the squatting males, preening themselves. A movement towards a male by a receptive female can spark the other males into action, scaring the female away (Hogan-Warburg 1966).

The choreography of an independent and a satellite on a double residence approached by a female consist of a pronounced performance of dominance by the independent. Circling, pecking, and feather pulling are endured by the submissive satellite, but only to a degree after which they flee the display territory or even the lek. The resident can mount the satellite and vice versa. Interference of copulation is seldomly observed although the copulating birds are surrounded by competing individuals, suggesting that a strong dominance hierarchy exists between the males (Widemo 1997).

If no interruptions occur the female approaches the chosen partner and crouches with the tail held up. Copulation occurs in a matter of seconds, especially when the male in question is a satellite, with the male mounting the female and lowering his wings, followed by a shake of feathers by the female.

Dominant independent males monopolize matings until a certain lek size is reached, after which marginal males are capable of acquiring a proportion of matings. After this optimal lek size is exceeded, the most dominant males may benefit more from leaving and displaying by themselves or conquering a territory from a smaller lek (Widemo 1997).

3.4 Mating

Both resident and satellite matings, and potential faeder interceptions, can occur on the same display territory. On average 89% of matings are by independent males (Hill 1991). Females rarely engage another partner after copulating with a resident (Hogan-Warburg 1966).

Lank *et al.* (2002) estimated that a minimum of 59% of females lay clutches with multiple fathers**.** Their rate of polyandry was found to be the highest out of all investigated lekking species and shorebirds. The role of post-copulatory sperm competition is thought to be of little importance in lekking species due to the usually low number of copulations and mates by females (Birkhead *et al.* 1987; Birkhead & Moller 1992). Species utilizing the lekking mating system also have relatively the smallest testes (Moller 1991). The ruff is an exception, having large testis and extremely large spermatozoa, nearly twice the length of the sperm of the large lekking grouses capercaillie (*Tetrao urogallus*) and black grouse (*Lyrurus tetrix*) (Ciereszko *et al.* 2009) and by far the largest in Scolopacidae (Table 1). The satellite and faeder males have up to 2,5 times larger testis than the independents (Figure 11), indicating high sperm production rate and large ejaculate volumes (Moller 1989). This can be a considerable advantage for these morphs that copulate infrequently and, unlike independents, cannot keep other males from mating with a female and are thus practically certain to face sperm competition. This may be among the key adaptations that have allowed the faeder and satellite morphs to eventually

proliferate and survive despite their considerably lower mating rates (Jukema & Piersma 2006; Küpper *et al.* 2016).

Table 1. Mating system, sperm size, body mass, testis length and egg sizes of members of Scolopacidae. Edited from Johnson & Briskie (1999).

Family/species	Mating system	Sperm length (μm)				Male (female) mass (g)	Testis length (mm)	Egg size index
		Total	Tail	Midpiece	Head			
Scolopacidae								
American Woodcock (*Scolopax minor*)	Polygyny	99.7	52.9	23.1	23.7	176.0 (219.0)	9.1	32.0
Common Snipe (*Gallinago gallinago*)	Polygyny	68.5	46.6	9.5	12.3	128.0 (116.0)	15.5	30.5
Whimbrel (*Numenius phaeopus*)	Monogamy	61.7	45.9	5.3	10.4	355.0 (404.0)	16.7	102.3
Short-billed Dowitcher (*Limnodromus griseus*)	Monogamy	62.8	45.2	6.0	11.6	111.0 (116.0)	9.0	34.5
Red Phalarope (*Phalaropus fulicaria*)	Polyandry	97.7	70.3	12.5	14.9	50.2 (61.1)	11.5	14.5
Lesser Yellowlegs (*Tringa flavipes*)	Monogamy	66.4	45.5	7.4	13.6	81.0 (81.0)	9.1	35.3
Stilt Sandpiper (*Micropalama himantopus*)	Monogamy	60.7	39.1	7.5	14.1	55.8 (60.4)	6.5	13.2
Ruff (*Philomachus pugnax*)	Lek	133.2	78.3	22.2	32.8	163.0 (95.0)	20.5	41.4
Semipalmated Sandpiper (*Calidris pusilla*)	Monogamy	62.5	43.3	8.2	10.9	28.1 (28.1)	9.2	22.5
White-rumped Sandpiper (*C. fuscicollis*)	Polygyny	68.8	—	—	—	44.9 (44.9)	10.3	19.6
Least Sandpiper (*C. minutilla*)	Monogamy	64.6	45.2	7.3	12.1	20.9 (20.9)	6.0	12.8
Pectoral Sandpiper (*C. melanotos*)	Polygyny	72.4	47.1	10.3	15.0	86.0 (60.0)	9.9	23.1
Dunlin (*C. alpina*)	Monogamy	68.3	45.6	8.9	13.8	54.7 (59.6)	7.8	28.2

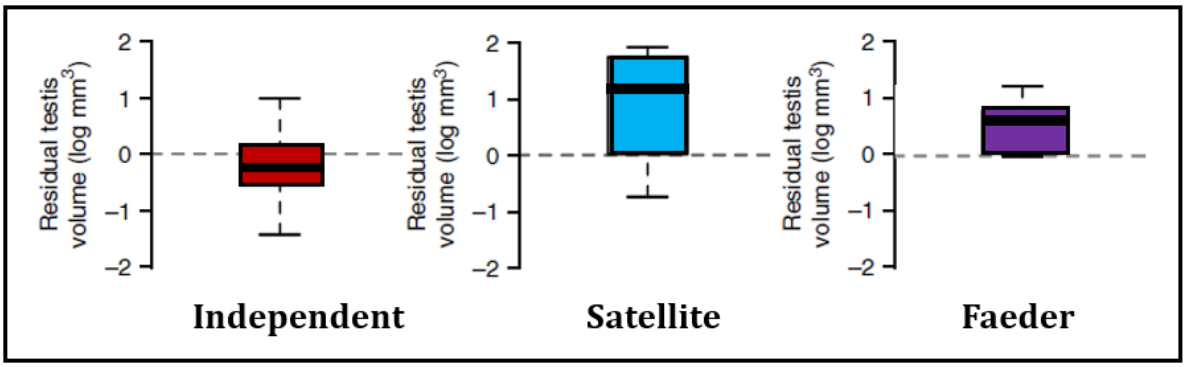


Figure 11. Residual testis size of male ruff morphs edited from Küpper *et al.* (2016).

Female avians are capable of storing sperm for considerable periods of time by utilizing specialized, blind-ended sperm storage tubules (Birkhead & Moller 1992). Motile sperm enters the tubule, where it can remain for days or several weeks (Hale 1955; Zenone *et al.* 1979) until emerging and proceeding towards the site of fertilization (Mero & Ogaswara 1970; Bakst *et al.* 1994). The details of the mechanisms by which the sperm is kept viable and released is still poorly understood, as is the degree of control that females may have on determining the outcome of sperm competition via regulation of the tubules. If females were found to be capable of favouring the sperm of certain males over others, it would introduce a new aspect in the reproductive behaviour of all birds and be of essential interest in the highly polygamic and competitive system seen in the ruff (Birkhead & Moller 1992).

3.5 Nesting

The female typically lays a clutch of 4 eggs singly in a space of 5-6 days from copulation. The nest is a shallow depression on the ground, hidden within tufts of grass. Incubation lasts for 20-24 days and the chicks are precocial, often leaving the nest within 24 hours of hatching given that the weather is favourable. The chicks immediately forage for themselves but depend on the mother for warmth. The sex-ratio of produced offspring appears to be connected to the physical state of the females, with females in good condition producing more female offspring **(Thuman *et al.* 2003).**

4.0 Key Questions

The reproductive behaviour of the ruff has been intensively examined through field observations, pedigree breeding and genetic investigations. Although research groups have spent multiple seasons observing the leks *in situ*, the projects have often been confined to a single lek and restricted by the amount of time observers have been able to remain at the sites and the precision of their observations.

In this thesis I describe the pilot study conducted on utilizing stand-alone cameras to record the lekking behaviour with by-the-second precision in the field. The method allows for accurate analysis of the numbers, morph frequencies, time use and behaviour of individually identifiable birds on several leks throughout the season. Cryptic features events, such as the presence of faeders, remain on tape and unlike real-time observations made in person, uncertain observations can be checked indefinitely.

The main topics of interest were the time investment of morphs and sexes, their influence on each other and the changes occurring as the season progressed. The following hypotheses were investigated:

1. The number of males on a lek influence the number of female visits. Female interest in a lek has been shown to increase with growing male numbers, therefore the number of males present should increase the number of female visits.

2. Tolerance of satellites by independents is suggested to be connected to their capability to attract females. This being true, one can predict that **presence of satellites increase number of female visits and their length of attendance on a lek.**

3. Satellites have been observed to opportunistically follow females. So **their presence is hypothesised to be influenced by female activity** and it can be predicted that satellite attendance on leks should correlate with female presence.

4. Satellite presence is influenced by independents. Satellites are able to utilize their strategy best at a lek that is large enough to attract females but on which the independents still tolerate them**.** Alternatively, to female presence being the influencing factor, the number of independents may increase the number and time allocation of satellites on a lek.

5. Female behaviour depends on time of the season. Indifferent behaviour by females, with visits ending without any interactions, are a common observation on a lek. This may be due to female fertility building up slowly before reaching a peak where visits become more intense with frequent copulations (Widemo 1996). This peak should be visible on the footage and computable from hatching dates.

6. Number of copulations depend on the number and devotedness of males. Females prefer large leks and especially residents that remain put on their display territories**.** Number of copulations should correlate positively with larger numbers and time allocation by males, which can be examined to an individual level through video.

5.0 Materials and Methods

5.1 Study sites

Field work was conducted from mid-May to the end of June in 2017 and 2018. In 2017 five shore meadow areas along the Bothnian Bay in Finland (Appendix 2) were monitored. Three of the sites were located on the mainland along the southern shore of Liminganlahti, 20-40km south/southwest from Oulu. Two were located on the southern and north-east shore of the island of Hailuoto, 25km west from Oulu.

Tauvonniemi (midpoint: *ETRS-TM35FIN: N 7191183,838, E 387819,089*), referred to as Tauvo, is a private protected area characterized by wet meadows and shifting sands.

Säärenperä (*N 7199207,068, E 403841,858*) holds three privately protected areas with extensive sedge meadows on the low-lying shore, fringed by deciduous forest.

Pitkänokka (*N 7194267,833, E 417840,054*) is a vast shoreline pasture of mainly short grasses where hundreds of cattle graze each summer. The area provides nesting habitat to multiple shorebirds, including black-tailed godwit (*Limosa limosa)* and a critically endangered subspecies of dunlin (*Calidris alpina schinzii).*

Tömppä (*N 7204909,500, E 396358,840*) on the southern portion of the island of Hailuoto is the largest shoreline meadow in the area and the adjacent Isomatala-Maasyvänlahti is the second most important bird water region in Finland.

Pökkö (*N 7217961,576, E 401054,567*) in the north-eastern portion of Hailuoto holds open, hay-growing shoreline meadows and a shoreline consisting of large mud flats.

In 2018 field work focused primarily on the Pitkänokka site on the mainland.

Each area contained a main lekking site, with male numbers continuously over 10 and multiple smaller side-leks with an average of 3-5 males. The areas had been investigated in 2016 by the Oulu University wader research group.

5.2 Lek recording and field observations

In 2017 filming was conducted on the main leks between 20.5 – 08.6 in each of the study areas. Small action cameras were placed on tripods at the main leks and filming started at 04:00 earliest and finished at 19:00 latest. Up to 4 hours of video was recorded on each session. Feeding flocks and individual birds in the shore meadows were followed with a Vortex Razor HD 27-60X85 birding scope to detect possible matings occurring outside of the leks.

The footage was investigated in the fall and winter of 2017 and 2018. Numbers of birds, their sex, male morphs and number of copulations were recorded. The arrival and departure of each individual was documented precisely, and mean values for time spent calculated based on sex, morph and in the case of independent males for residents and marginals. The coloration of each male was recorded and used for individual identification. A continuous record of behavioural observations was kept.

In 2018 the leks were observed on location using a birding scope between 12.5 – 07.6, starting at 05:40 earliest and finishing at 12:45 latest, for a total of 2,025 minutes. The number of birds and ratio of male morphs were recorded by scan samples at 15-minute intervals. Each copulation and the individuals involved were recorded.

5.3 Nest location and bird handling

Nest location focused in the Pitkänokka study site in both years due to high number of nesting females and accessibility of the area. Nest sites were located by flushing out females and tracing the take-off site, which were carefully approached to prevent trampling the nest. Height and width of the eggs were measured, and the eggs were floated to estimate hatching dates. The nests were given a number, which also functioned as an individual code for the female, and the location was recorded on a Garmin GPSMAP 64s. To prevent leaving scent or visual cues for predators, the nests were never approached directly and discreet natural markers in the form of rocks, wood and crosses made of grass were used to aid in relocating the nest in later visits. Total number of nests located and monitored was 72 in 2017 and 88 in 2018.

Walk-in cages were placed over the nests and to passively capture the females. The trapped bird was ringed with a metal ring and a unique colour ring combination. Wing, tarsus, bill and cranium length and weight were recorded. Blood samples were extracted from the basilic vein using a cauge needle and stored in a sterile Eppendorf tube containing Longmire buffer that ensured the viability of the sample until later cold storage. After ensuring the blood flow had dried up, the bird was released. The nests were visited on the calculated hatching date to prevent the nidifugous chicks from disappearing before sampling and. Due to inability to determine chick sex, all juveniles were assumed male and ringed with the larger metal BT ring. No colour bands were placed on chicks. Due to underdeveloped wings, the blood samples were taken from the metatarsal vein.

The hatching data was used to calculate the time of laying of the first egg and the beginning of incubation after laying of the last egg (5 days to lay 4 eggs and additional 26 days for incubation, Lank 2002). This allowed the date when reeves left the mating pool inferred and reconstruction of a mating pool for the sample population.

The ruff observation data for 2017 and 2018 was acquired from the Birdlife Finland's Tiira database (Birdlife Finland 2019). Ruff observations from 3 highly active observational areas, Siikajoki, Oulu and Hailuoto (Appendix 3), were taken and combined to gain the maximum number of birds observed during the time period between 28.4 – 11.6.

5.4 Statistical analysis

The 2017 video observations were transformed into quarter-hour readings by calculating the birds present on each 15-minute period of footage for compatibility with the 2018 observational data. The quarter-hour data was analysed using RStudio (version 1.1.463)

Selection gradient analyses were utilized with Zero-Adjusted Inverse Gaussian models (Heller *et al.* 2006) by using the GAMLSS ("*Generalized Additive Models for Location, Scale and Shape*") function (Rigby & Stasinopoulos 2005; Stasinopoulos *et al.* 2017) in R (R Core Team 2013) for the ability to deal with heterogeneity, skewness and zero-inflated data, such as female minutes and copulations. Datasets included a large amount of 0-values due to the seldom, short-lasting visits of the females in contrast to the constant presence of males on a lek. The data from 2017 and 2018 was analysed as pooled together.
Linear regression was used to assess the minutes spent by independents, satellites and females on the leks in relation to date and to the % of females within the mating pool.
The relationships between the number of ruffs present according to male morph and sex, date, filming session length and time of day were investigated via correlation analysis and significance tests using the R corrplot package (Wei *et al.* 2017). The factors checked for correlation were the filming date, session (*early* 04:00-08:00, *mid-day* 08:00-12:00 and *late* from 12:00 onwards), time of day (*hh:mm*), male morphs and females. For Tauvo and Säärenperä 2017 the independent males were investigated as both together as "independents" and separately as residents and marginals.

6.0 Results

6.1 Weather

There were notable differences in the weather conditions and progress of spring between 2017 and 2018 (Figure 12). May of 2017 was exceptionally cold whereas in 2018 the conditions were notably warm. In 2017 ruffs began arriving at the coastal area on 4 May with peak numbers observed on the 21-25 May. First arrivals of 2018 were observed on the 7 May with peak numbers appearing between 13-17 May.

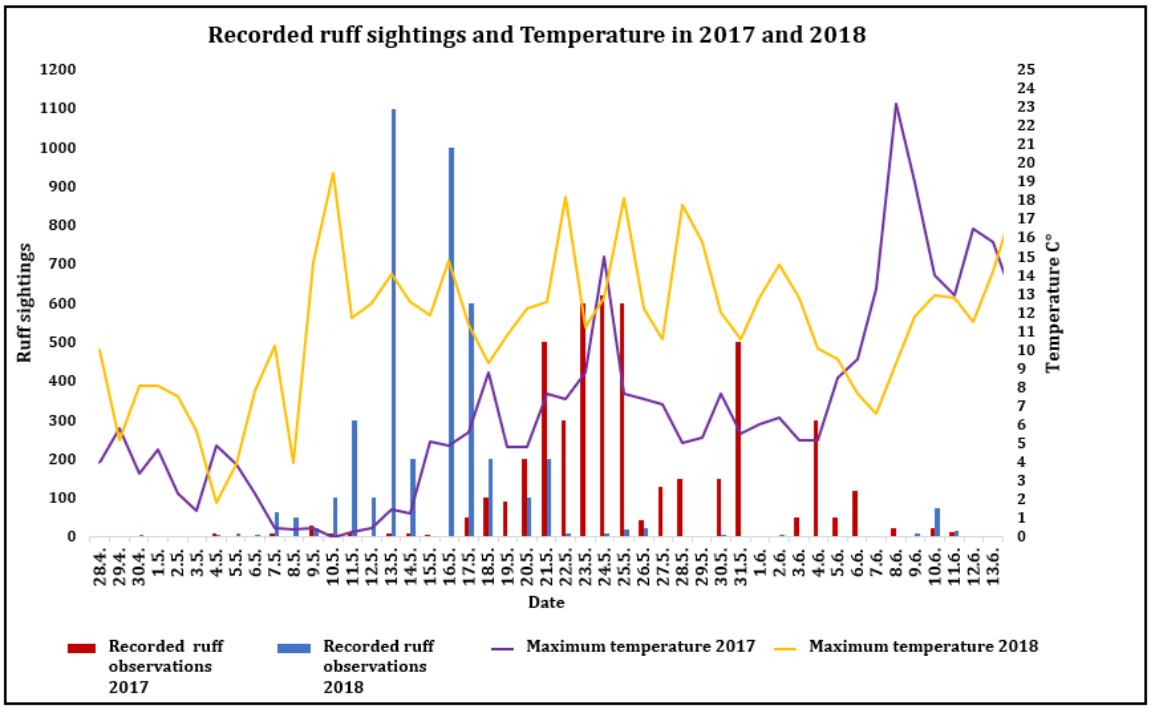


Figure 12. Number of ruff observations in the Siikajoki-Oulu-Hailuoto area (Birdlife Finland 2019) and archived maximum temperatures recorded at the Vihreäsaari weather station in Oulu (Finnish Meteorological Institute 2019) between 28 April – 13 June 2017 (red bars, purple line) and 2018 (blue bars, yellow line).

Reeves in the study areas had been ringed and colour banded in 2016, during which 31 females were banded.

A total of 18 nests out of the 72 (25 %) monitored in 2017 were lost to predation and trampling by cattle. A total of 48 females and 132 chicks were ringed.

In 2018 there were 33 nests out of 88 (37.5 %) lost due to predation and trampling. A total of 50 females and 183 chicks were ringed. Out of the 2016 females 2 were observed in 2017. A total of 14 banded females were controlled in 2018, out of which 2 had been banded in 2016 but not seen in 2017.

6.2 Lek and field observations

A total of 19 sessions were filmed, yielding 3,810 minutes of footage (Table 2). Monitoring of flocks in the shore meadows yielded no observations of matings occurring outside the leks. Occasionally a singular or few males were seen performing actions loosely resembling those seen in leks in the vicinity of feeding female flocks, and a single event of an independent performing with a satellite to feeding females was observed in Tauvo. This, nor any of the other occasions, provoked any response from the females. Very few copulations were observed in 2017 whereas in 2018 a notable streak of copulations occurred in rapid succession (Table 3).

Table 2. Location, period of filming and amount of footage obtained from major leks in 2017.

Location	Filming period	Footage (*hh:mm:ss*)
Tauvo	21-29.5.2017	*18:00:00*
Säärenperä	23.5 - 8.6.2017	*22:30:00*
Pitkänokka	22.5-24.5.2017	*6:15:00*
Tömppä	30.5 - 04.6.2017	*6:00:00*
Pökkö	27.5-6.6.2017	*10:45:00*

Very few copulations were observed in 2017 whereas in 2018 a notable streak of copulations occurred in rapid succession (Table 3), a majority in a single session of observations on 16.5 which saw continuous female attendance throughout the observation period (Figure 16).

Table 3. Number of observed copulations at each lek site in 2017 and 2018 by resident and marginal independents and satellite males.

Location	Observation period	Resident	Marginal	Satellite
Tauvo	21-29.5.2017	4	0	1
Säärenperä	23.5 - 8.6.2017	0	0	2
Pitkänokka	22.5-24.5.2017	0	1	0
Tömppä	30.5 - 04.6.2017	0	0	0
Pökkö	27.5-6.6.2017	0	0	0
Pitkänokka 2018	12.5-28.5.2018	28	1	8

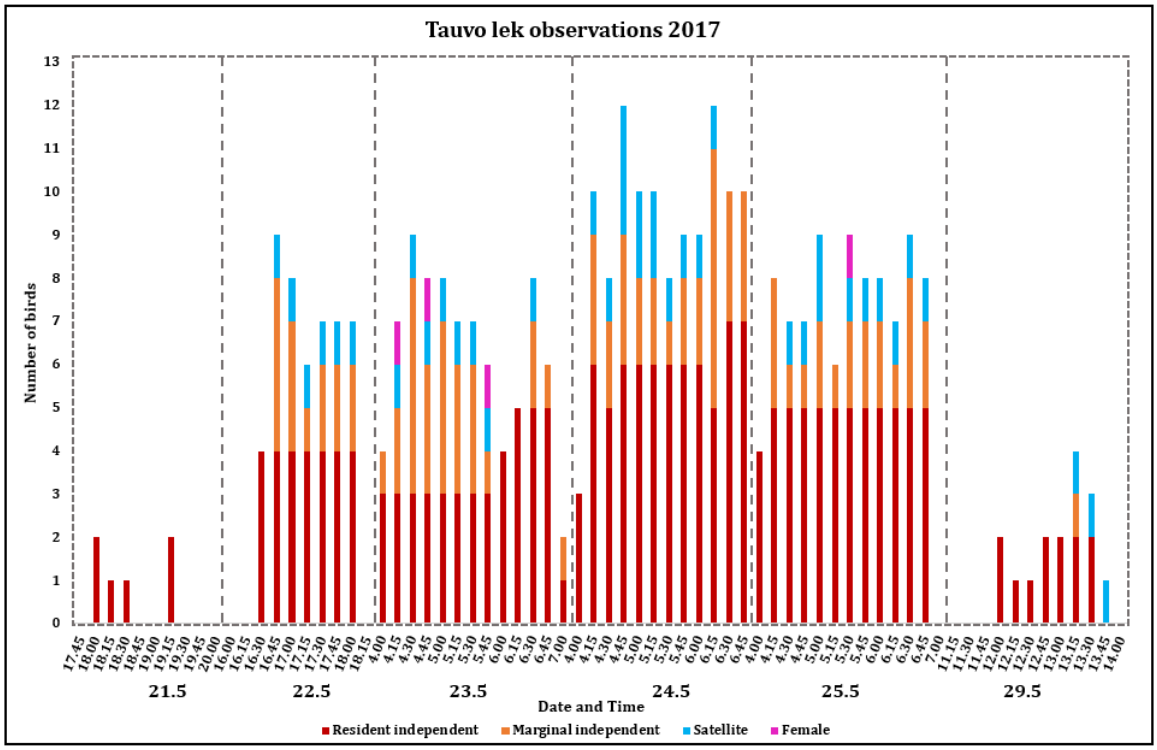


Figure 13. Observations of resident independent (red), marginal independent (orange), satellite (blue) and female (pink) ruffs in the Tauvo (*N 7191183,838, E 387819,089*) lekking site in May 2017. Time of day (*hh:mm*) and date presented below the x-axis, days separated by dashed lines.

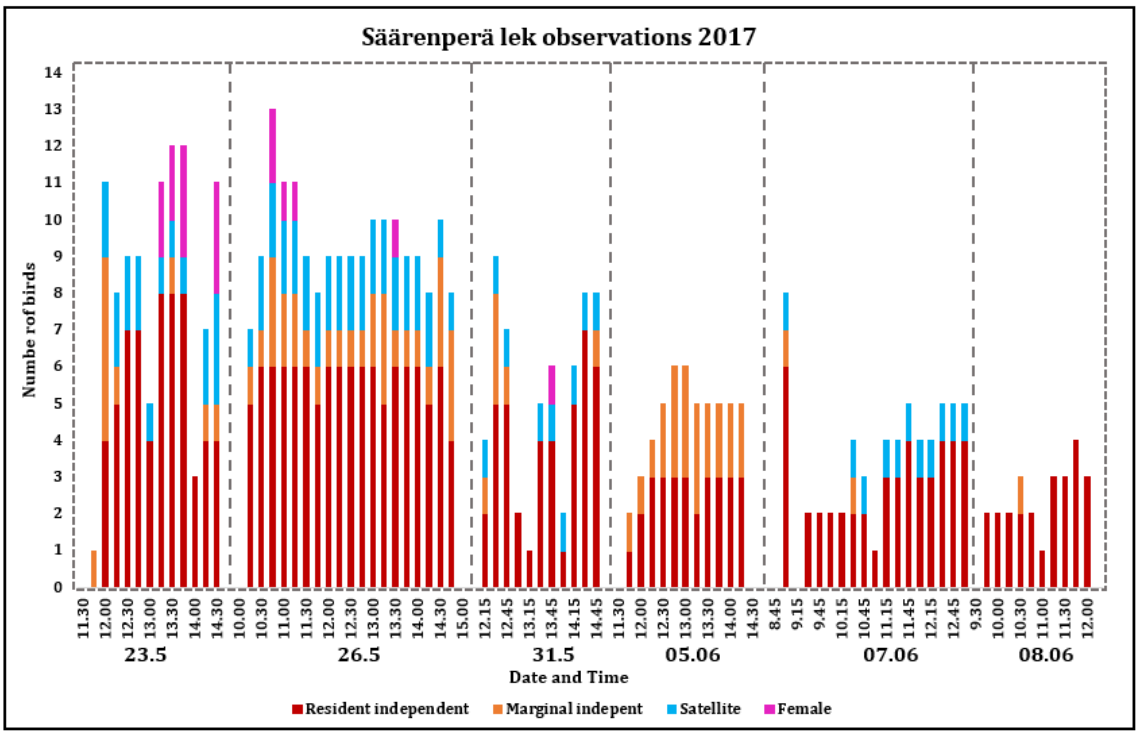
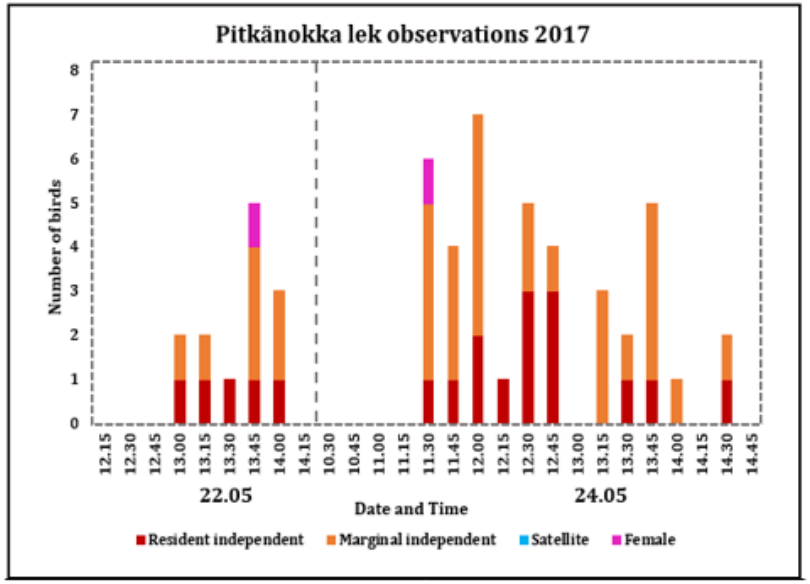
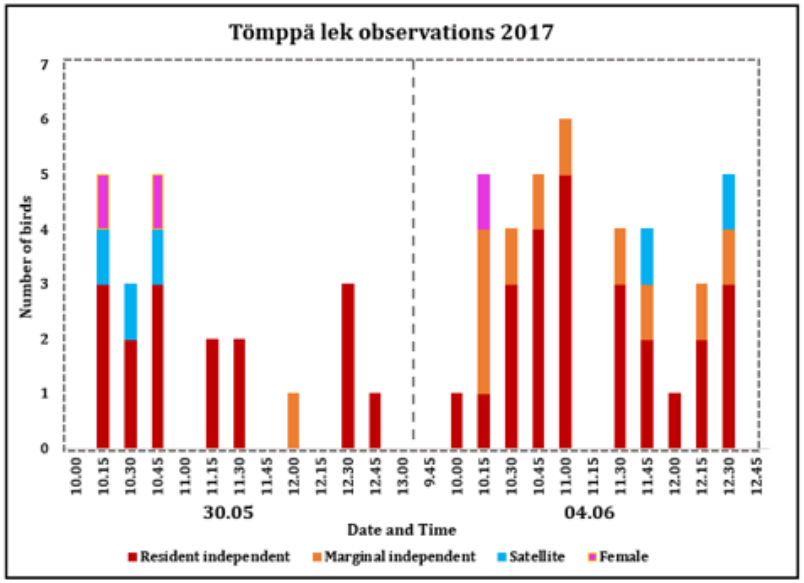


Figure 14. Observations of resident independent (red), marginal independent (orange), satellite (blue) and female (pink) ruffs in the Säärenperä (N 7199207,068, E 403841,858) lekking site in May 2017. Time of day (hh:mm) and date presented below the x-axis, days separated by dashed lines.





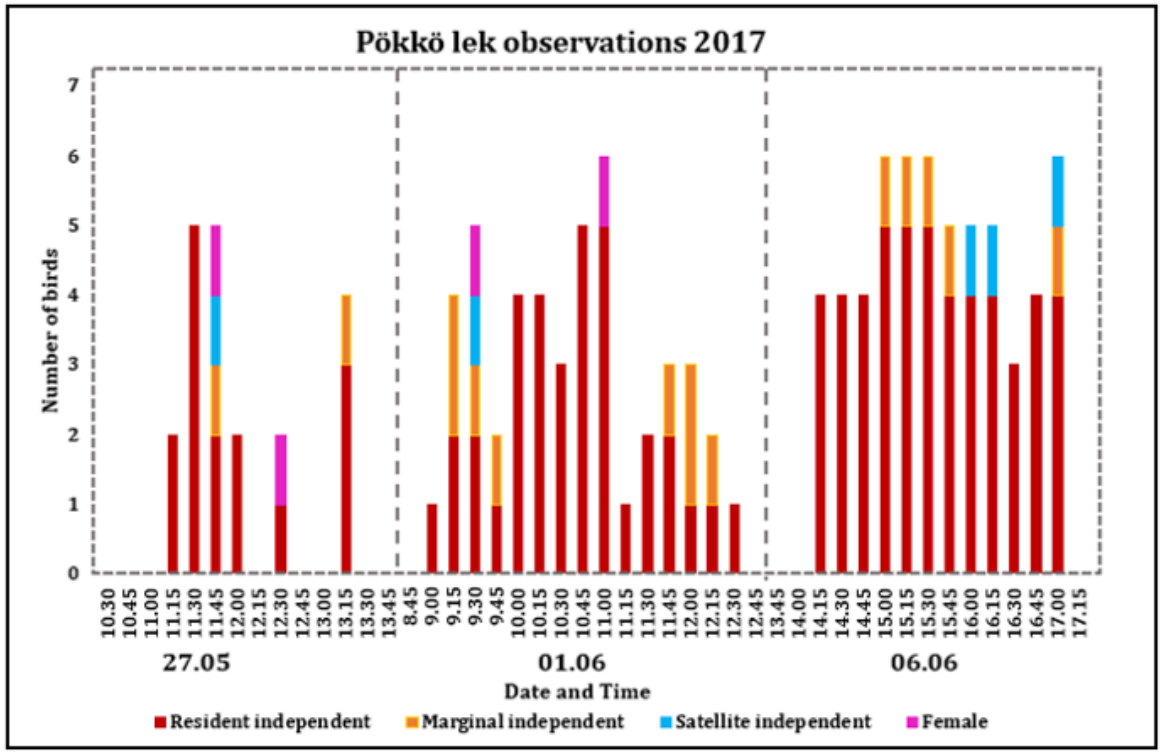


Figure 15. Observations of resident independent (red), marginal independent (orange), satellite (blue) and female (pink) ruffs in the Pitkänokka (*N 7194267,833, E 417840,054*), Tömppä (*N 7204909,500, E 396358,840*) and Pökkö (*N 7217961,576, E 401054,567*) lekking site in May and June 2017. Time of day (*hh:mm*) and date presented below the x-axis, days separated by dashed lines.

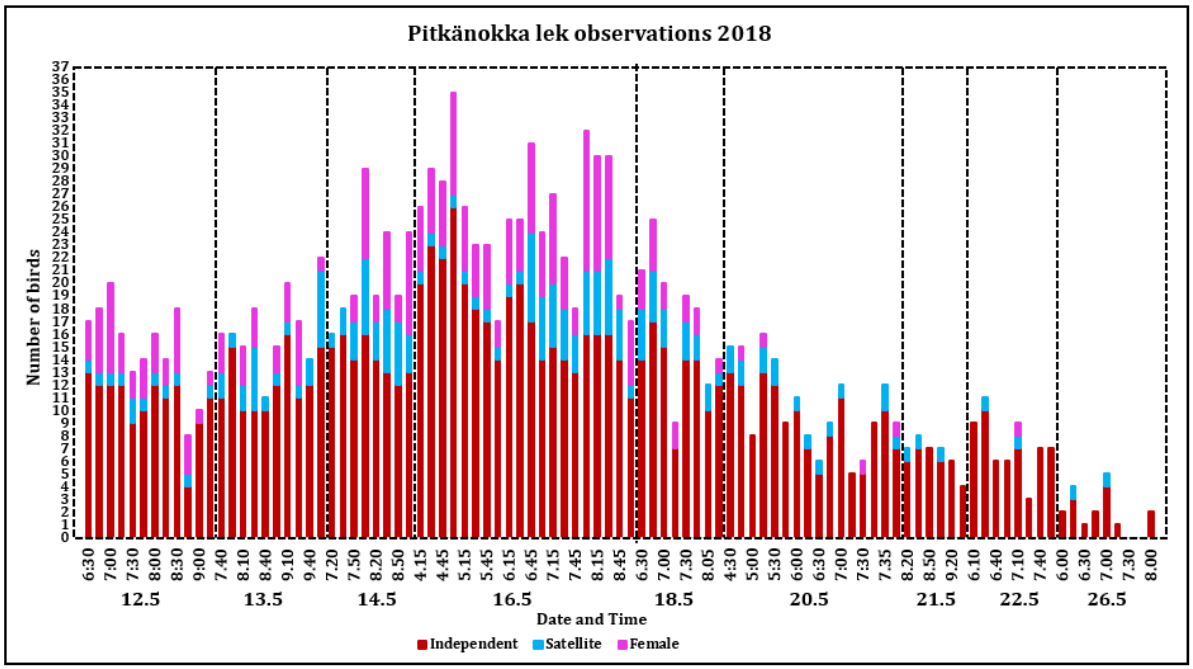


Figure 16. Observations of independent (red), satellite (blue) and female (pink) ruffs on the Pitkänokka (*N 7194267,833, E 417840,054*) lek site in May 2018. Time of day (*hh:mm)* and date presented below the x-axis. Days separated by dashed lines.

The independent males were divided into residents and marginals in investigation of the 2017 video footage (Figures 13-15), due to ease of long-term recognition of male status from the recordings. Although male status was identified in the field observations of 2018 it was not done to the same individual degree, leading to the males being represented as single "independent block" in the 2018 data (Figure 16).

6.3 Receptiveness and time use

The number of ruffs and the time spent on the leks had a clear relationship with the progression of the season (Figure 17-18). Back-calculation from hatching dates of monitored nests yielded a clear peak in the % of receptive females from the sample population (Figure 17). The time allocated to lek attendance by both independent and satellite males, as well as females, was found to have a linear relationship with the % of females in the mating pool (Figure 18).

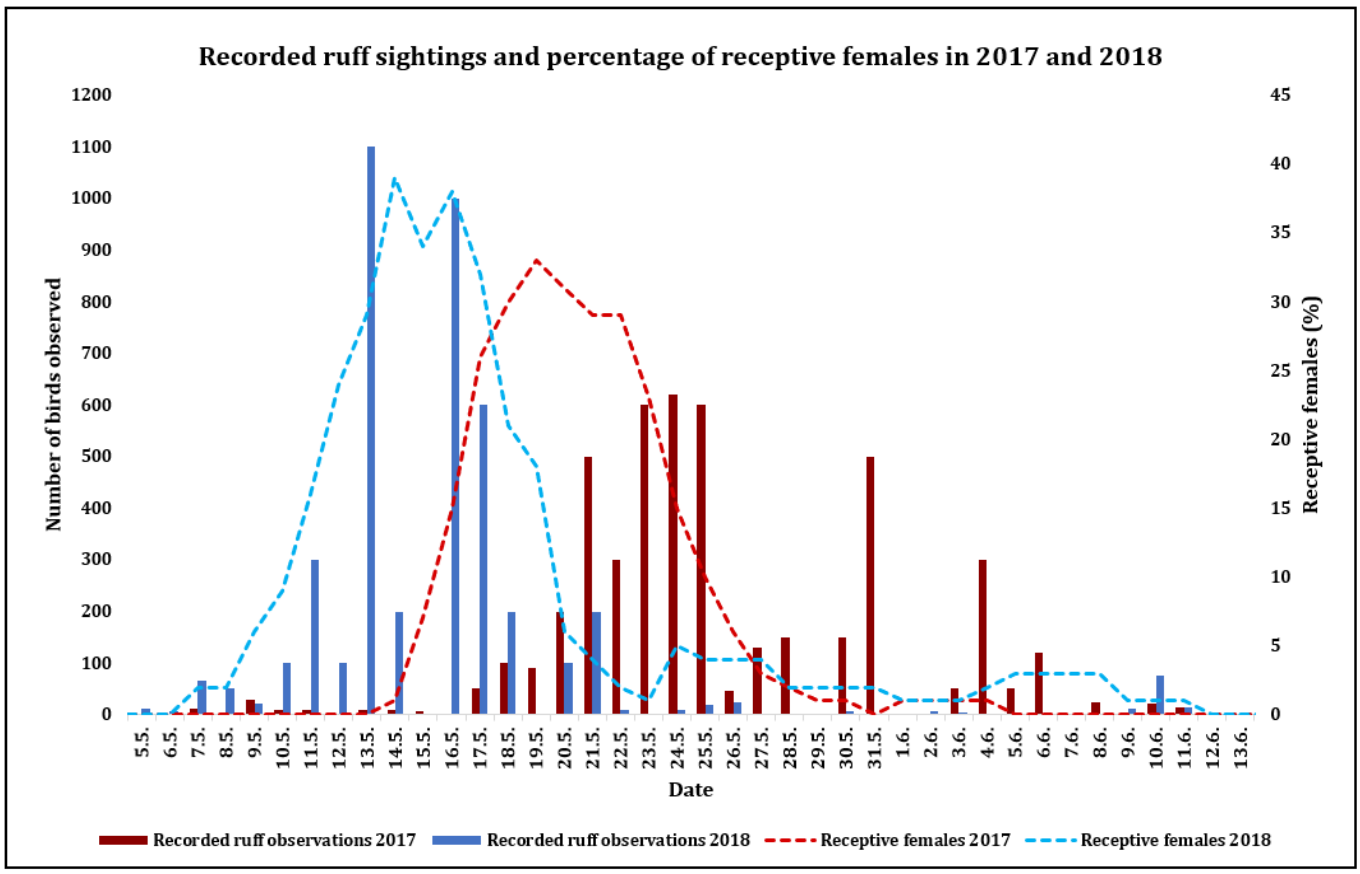


Figure 17. Percentage of receptive ruff females (Dashed lines, Red = 2017, Blue = 2018.) back calculated from monitored nest hatching dates (5 days to lay 4 eggs and additional 26 days for incubation, **Lank 2002**) in the Pitkänokka nesting site (*N 7194267,833, E 417840,054)* and number of recorded ruff observations during the spring migrations of 2017 (red bars) and 2018 (blue bars) from the Hailuoto-Siikajoki-Lumijoki area. Female receptiveness back calculated from hatching dates of monitored nests (5 days to lay 4 eggs and additional 26 days for incubation). Observation data obtained from Birdlife Finland's Tiira database (Birdlife Finland 2019).

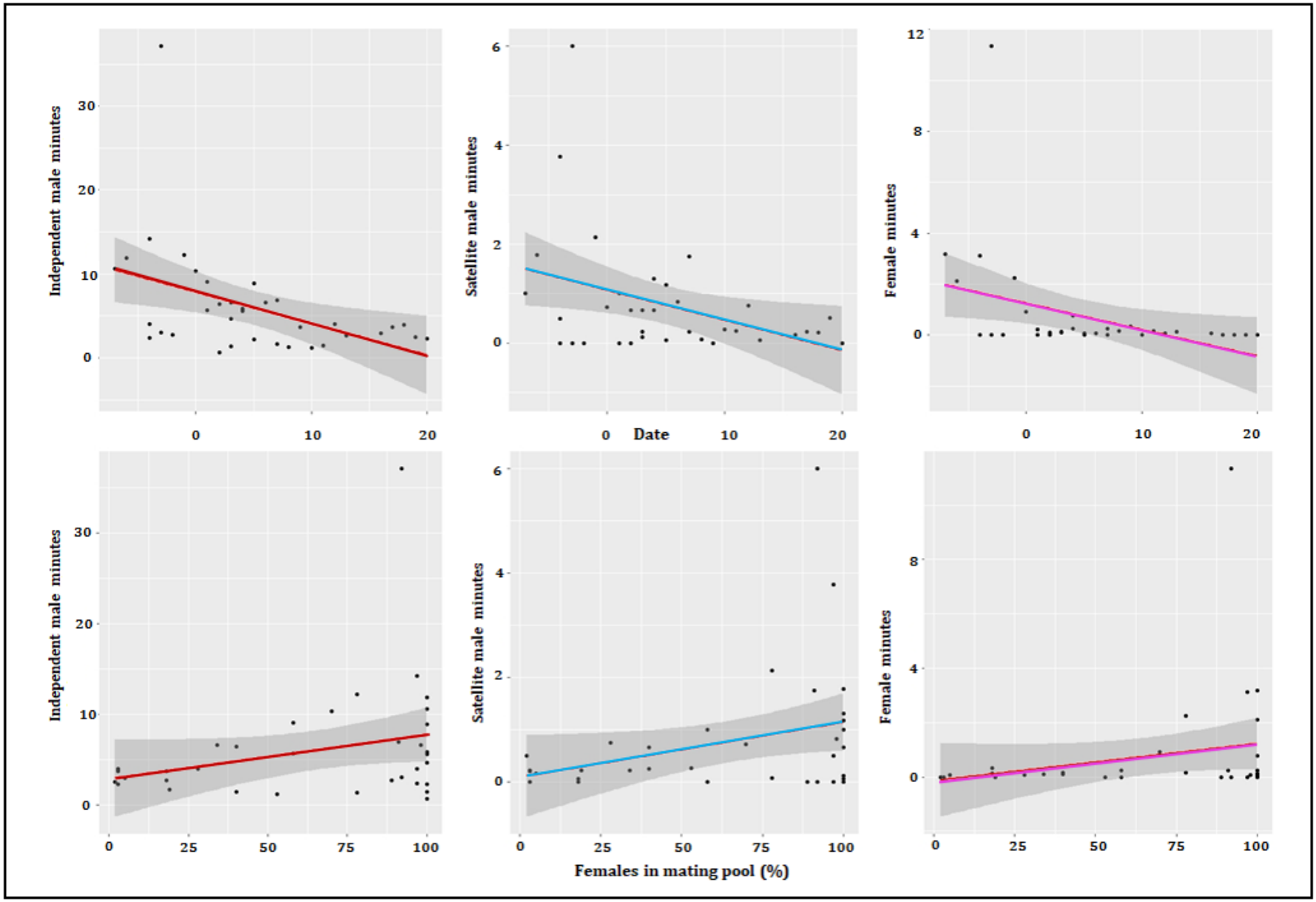


Figure 18. Linear regression analysis of minutes spent at leks by independent males (red), satellite males (blue) and females (pink) in relation to date (top row) and to percentage of females within the mating pool back calculated from hatching dates of monitored nests (5 days to lay 4 eggs and additional 26 days for incubation) (bottom row). Each dot represents minutes spent by an individual at a lek at a given time.

6.4 Correlations between morphs, sexes and temporal variables

Tauvo and Säärenperä provided the most video footage and were monitored for the same consecutive days in 2017 (Table 2, Figure 13-14). Them, along with Pitkänokka lek in 2018 (Figure 16) allowed for use in correlation analysis (Figure 19).
For Tauvo and Säärenperä males were allocated into residents or marginals from the video material. This division was not done in Pitkänokka. Correlations between the date, session and time of day variables are due to their temporal dependence. Both numbers of resident and marginal independents, satellites and females showed a negative relationship with date, session and time of day, except in Tauvo where there was no correlation between the temporal variables and numbers of ruffs of any type. A positive relationship was found to exist between numbers of residents and marginals, as well as residents and satellites. Marginals and satellites also displayed a positive correlation as well as independents pooled as one and satellites. Females showed a positive correlation with independents and satellites, except in Tauvo where no significant relationships were found.

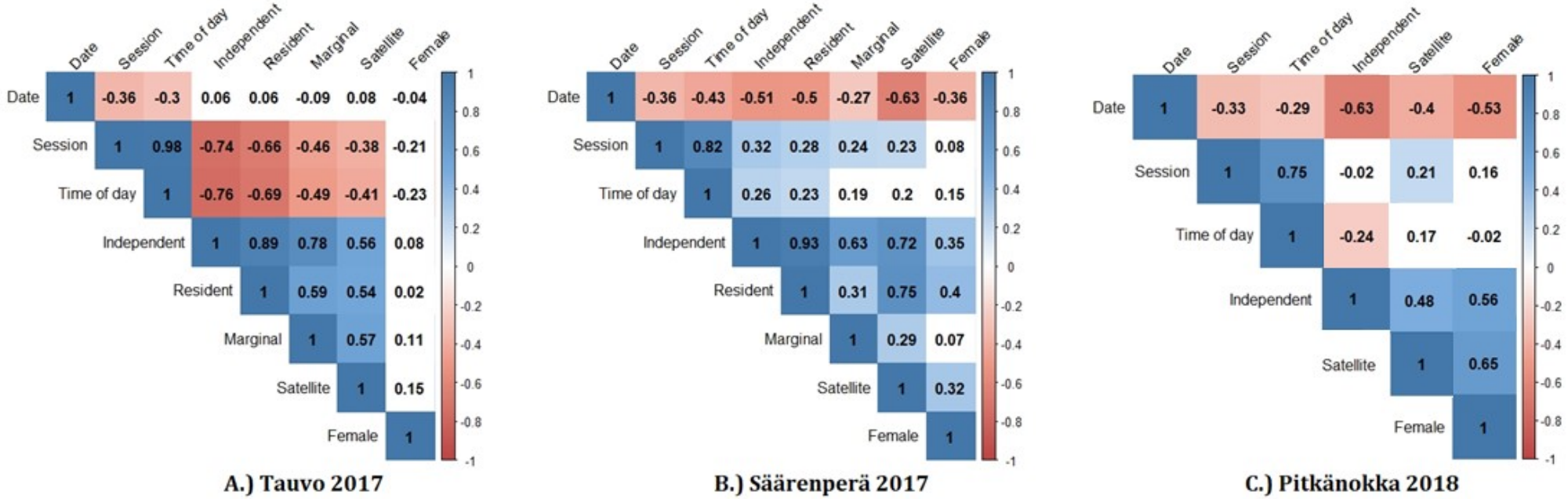


Figure 19. Correlograms of numbers of male morphs and female ruffs in relation to observation date, filming session (early, midday, late), time of day (hh:mm) and to each other from three lek sites: **A.)** Tauvo (21.5 – 29.5.2017, 18 hours of footage), **B.)** Säärenperä (23.5 – 08.06.2017, 22,5 hours of footage) and **C.)** Pitkänokka 2018 (13.5 – 29.05.2018, 31 hours of observations). The Intensity of coloration corresponds with higher correlation coefficients. Blue = Positive correlation Red = Negative correlation, White = non-significant correlation (p = >0.05).

6.5 Lek attendance and copulation frequency

In the results tables of the selection gradient analysis the Mu coefficient interprets the portion of the data with values larger than 0, whereas the Nu coefficient describes the relationship of the inserted variables to the 0 values.

Table 4. Results of GAMLSS analysis of female minutes (time spent at the lek) in relation to Satellite and Independent male minutes.

Female minutes in relation to Satellite and Independent minutes				
Mu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	-2,46944	0,23135	-10,674	$2{,}24^{-11}$
Satellite minutes / Recorded minutes	0,27884	0,43635	0,639	0,528
Independent minutes / Recorded minutes	0,18793	0,07306	2,572	0,0157
Nu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	0,8237	0,7432	1,108	0,277
Satellite minutes / Recorded minutes	-1,5597	1,3955	-1,118	0,273
Independent minutes / Recorded minutes	-0,139	0,2191	-0,634	0,531

Independent male minutes spent on lek increased the female minutes (p=0,0157) spent on lek (Table 4), whereas satellite minutes did not (p=0,0528).

Table 5. Results of GAMLSS analysis of copulations per hour in relation to Satellite and Independent male minutes

Copulations per hour in relation to Satellite and Independent minutes				
Mu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	-0,5611	0,4108	-1,366	0,1828
Satellite minutes / Recorded minutes	-1,0979	0,4829	-2,227	0,0341
Independent minutes / Recorded minutes	0,2605	0,1287	2,023	0,0527
Nu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	2,8947	1,1208	2,583	0,0153
Satellite minutes / Recorded minutes	-4,2546	2,0023	-2,125	0,0426
Independent minutes / Recorded minutes	0,1962	0,2751	0,713	0,4816

Satellite minutes affected number of copulations (p=0,0341) and also the lack of copulations (p=0,0426) although only at a barely significant level (Table 5).

Table 6. Results of GAMLSS analysis female minutes in relation to Time of Day (Early: *04:00-08:00*, Midday: *08:00-12:00*, Late: *12:00* onwards) and to Size of Lek (Small: <10 individuals, Large >10 individuals).

Female minutes in relation to Time of Day and Size of Lek				
Mu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	-2,9068	1,8732	-1,552	0,132
Time of Day	-0,4756	0,453	-1,05	0,3027
Size of lek	1,8774	0,8134	2,308	0,0286
Nu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	-0,2489	1,5394	-0,162	0,873
Time of Day	0,2964	0,4329	0,685	0,499
Size of lek	-0,474	0,7502	-0,632	0,533

Table 7. Results of GAMLSS analysis of satellite minutes in relation to Time of Day (Early: *04:00-08:00*, Midday: *08:00-12:00*, Late: *12:00* onwards) and to Size of Lek (Small: <10 individuals, Large >10 individuals).

Satellite minutes in relation to Time of Day and Size of Lek				
Mu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	-2,7557	0,6644	-4,147	0,0002
Time of Day	-0,3473	0,1718	-2,021	0,0529
Size of lek	1,7403	0,3426	5,08	$2,23^{-5}$
Nu Coefficients	**Estimate**	**Std.Error**	**t-value**	**p-value**
Intercept	0,3157	1,8507	0,171	0,866
Time of Day	-0,339	0,5722	-0,593	0,558
Size of lek	-0,6936	0,8853	-0,783	0,44

Time of day did not influence the amount of female (p=0,3027) nor satellite minutes (p=0,0529) spent on leks, but both females (p=0,0286) and especially satellites ($p=2,23^{-5}$) were found to be positively influenced by the size of the lek (Table 6, Table 7).

7.0 Discussion

7.1 Progress of lekking

The differences between the progress of spring in the two years of observations had a prominent effect on the arrival and lekking of ruffs, with the season of 2018 beginning nearly 2 weeks earlier than in 2017. Females reached peak receptiveness a week earlier in 2018 and (Figure 17) and there was also a 10-day difference in peak hatching dates between the years (Appendix 1).

Activity on the leks peaked between 04:00 and 07:00 (Figures 13-16) after which the leks remained active with a slow decline, until a second peak occurred between 14:00 and 17:00. Resident males were present on the leks almost continuously. A varying flow of marginals occurred throughout the day, with considerable individual differences in persistence. The more determined marginals may have been on the lookout for potential resident positions. Satellites appeared on the leks later than independents, although in Tauvo and Pitkänokka a satellite was present almost throughout a day. The central-peripheral satellite split was visible in occasions when multiple satellites occupied the lek simultaneously. In 2017 most female visits were recorded between 10:00 and 13:00, whereas Pitkänokka 2018 saw higher numbers of female activity in the early hours of the day. In Tauvo 2017 residents, marginals and satellites showed negative correlations with session and time of day, whereas in Säärenperä 2017 the correlations with the temporal variables were slightly positive (Figure 19). This may rise from the difference in filming times, footage from Tauvo was mainly from early in the morning, between 04:00 – 12:00 whereas the material from Säärenperä was from 09:00 – 14:00. The two leks appear to exhibit two different situations of the lek, Tauvo shows the morning peak falling and Säärenperä displays rising towards an afternoon peak. The stronger negative correlation of residents to session (-0.66) and time (-0.69) than the marginals (-0.46 and -0.49) could indicate that the residents time their feeding visits accordingly to the moment of decreased activity between the morning and afternoon peaks.
Each monitored lek experienced the activity peaks at slightly different times. This could potentially rise from females, and the marginals and satellites trailing them, moving from adjacent lek to lek, although it is unlikely that large numbers of birds would move through all the monitored sites. The timing of activity by sex and morph fall in line with the thorough observations of Hogan-Warburg (1966) and Lank & Smith (1987).

In 2017 the number of females and copulations remained low on each monitored lek. Back-calculations from hatching dates showed that the receptiveness peak occurred on 19.5 (35% of females) and remained at over 30% until a steep drop after 22.5. The number of birds and their time spent on the leks began falling steadily after few peak days on each lek (Figures 13-15). This however does not coincide with the largest numbers of observed birds (Figure 12), suggesting that the majority of observed ruffs may still have been migrating up north and only a small proportion remained in the Oulu region, although the voluntary nature of the Tiira database makes the observational data directional. Since earliest video footage was filmed in Tauvo on the 21.5 the filming began at the point of the season when the numbers of receptive females began rapidly reducing, providing an explanation for the relatively low numbers observed.

On the Pitkänokka lek in 2018 the peak number of independents, satellites and females observed coincides with the highest number of females concurrently in the mating pool (37%) (Figures 16-17) on 16.5. A record number of 17 independent and 7 satellite copulations were observed on this peak day. On the consecutive days the numbers of females and satellites sharply declined, with a less dramatic, steady drop in the numbers of independents.

Both the receptiveness (Figure 17) and hatching (Appendix 1) peaks occurred within a few successive days, implying synchrony of the reproductive cycle. Possible selective advantages conferred to the ruffs may come in the form of reduced predation pressure through satiation of predators or peak availability of insect food the juveniles depend on (Findlay & Cooke 1982).

7.2 Influential relationships

The falling linear trend and negative correlations (Figures 18-19) with temporal variables by all ruffs regardless of sex and morph were expected. The number of female visitations steadily fell after the receptiveness peak as more females began incubating, making lek attendance progressively less beneficial for both independents and satellites. The amount of satellite males present fell at a slower rate, followed by the independents which remained the most steadfast. The eventual reduction of independent numbers is likely also connected to the residents exhausting their energy reserves and being unable to continue exclusively attending the leks. In the case of marginals, it is probable that they moved north, where lekking takes place gradually later than in the southern areas**.** It would appear improbable that residents would follow a similar pattern due to their considerable resource investment, and unlikelihood of being able to assume a dominant position at a completely new lek.

The lack of female relationships (Figure 19) with any other variable in Tauvo in 2017 was unexpected, although it is likely explained by the low numbers of female observations. Both Tömppä and Pökkö saw only short, individual female visits. Säärenperä 2017 and especially Pitkänokka 2018 experienced more numerous female visitations (Figure 16). In Tauvo females were observed in multiple feeding flocks but there were very few female visits on the lek during the whole filming period. This is likely affiliated with passing of the receptivity peak before filming was initiated and the already copulated females focusing on feeding to lay eggs and incubate. It is possible that the receptivity of females at different lekking sites fall at different paces, explaining the different rates of decline in female visitations at different areas. Differences in the suitability of proximate areas for nesting may play a crucial role in the initial number of females occurring near a given lek (Widemo 1997).

The possible central role of resident males was underlined by the correlation analysis. Marginals showed a positive relationships with residents in Tauvo (0.59) and Säärenperä 2017 (0.31). Females displayed a clear positive correlation for residents (0.4) and satellites (0.32) over marginals (0.07) in Säärenperä 2017. In Pitkänokka 2018 there was a positive relationship between females and independents (0.56) grouped together and even more to satellites (0.65). Satellites showed a clearly stronger relationship to resident numbers (0.75) than marginals (0.29) at Säärenperä 2017, but at Tauvo the correlations were similar (0.54 and 0.57 respectively).
The selection gradient analyses with GAMLSS (Tables 4-7) showed a significant relationship between female minutes spent on the lek and independent male minutes, as well as a significant relationship between satellite minutes and the number of observed copulations (Table 5). This would suggest that the number of residents functions as the foundation of lek formation, drawing in marginals and producing an attractive lek to females and satellites alike. Although the role of residents is fundamental, their success is positively affected by numbers of marginals due to the increased “value” of a larger lek. The relationship between satellites and females appears to be reciprocal, producing a situation where attracting both satellites and females is beneficial to a lek or showing that the movements of the two are often connected. The intriguing possibility of satellites playing a role in influencing female receptivity would further highlight their special position in composition of a ruff lek (Figure 20).

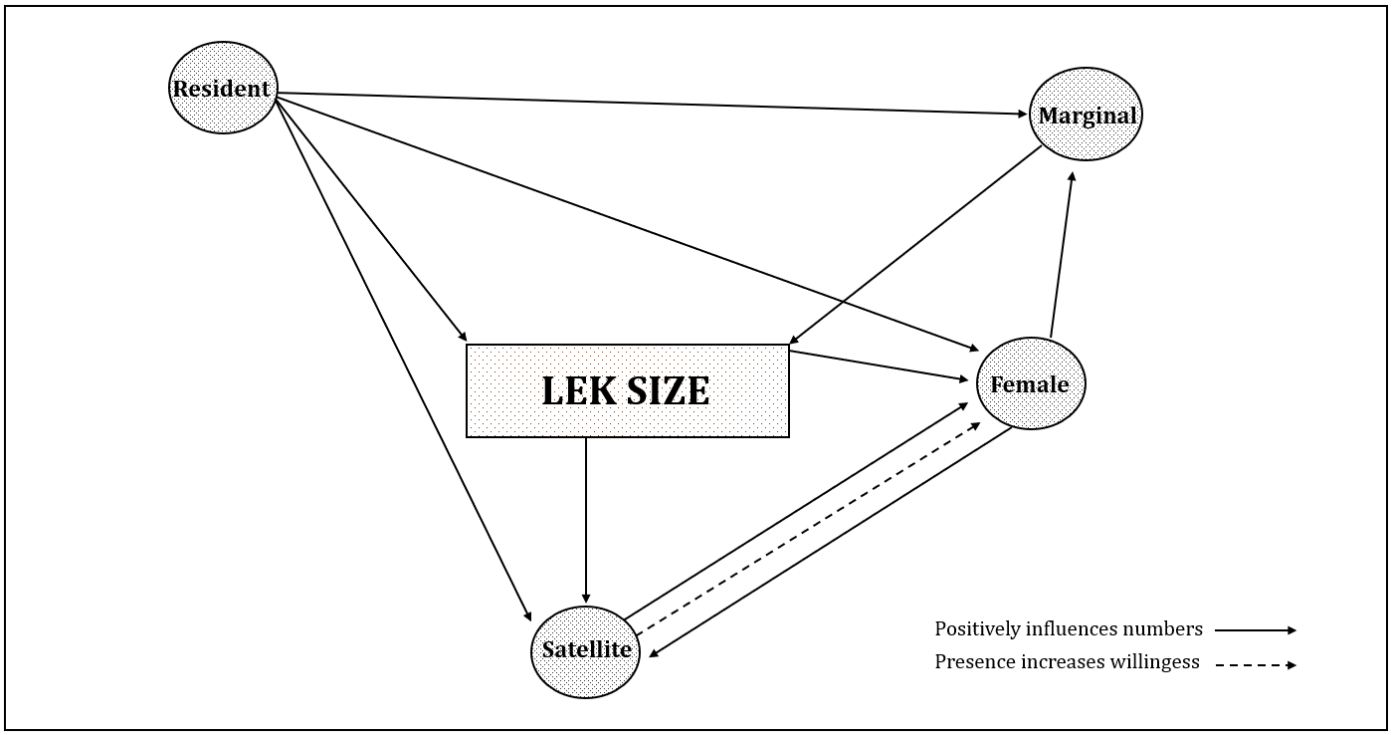


Figure 20. Chart of influential relationships between ruff male morphs, sexes and lek size. The positive influences (solid arrows) indicate that increased number of individuals in the group (Resident/Marginal/Satellite/Female) on lek increase the numbers of individuals in the target group. The residents and marginals form the major numbers of the lek, and increased lek size positively affects the numbers of females and satellites. Satellites influence the female willingness to copulate (dashed arrow) with both themselves and residents.

Near the end of the season aggregations of males into assemblances resembling leks were observed in Pitkänokka in 2018 (K. Koivula, personal communications, September 24, 2019). This may represent a last-chance attempt of remaining marginal males, and perhaps residents that have managed to recover from the main lekking, to attract and mate with females that have not yet copulated or attempt a second clutch after losing the first.

7.3 Decisions influencing time and resource allocation

A complex set of decisions must be made by individuals to determine if allocating time and energy to a lek is a worthwhile investment. The fundamental "questions" depend on their sex, morph and status and are further influenced by factors like age and physical fitness of both the individuals and their competitors.

These factors underlie the division of independents into residents and marginals. The marginal independents attain very few matings (Widemo 1997) and most of their time on the leks is unprofitable for them. Although it is possible that the marginal status is temporary, for individuals that are not able to attain resident status on a lek the optimal time use is crucial.

Preliminary tracking experiments have indicated that some independents travel vast distances in search of leks (D Lank 2019 personal communication June 22), progressively moving north with the season until the northernmost leks have died down. It is most likely that the males engaging in such an enormous undertaking would be marginals. Two potential manners of operation may exist in the marginals, the first being the "remainers" that linger at leks on a relatively limited area and persist until they are able to secure a place as a resident. The second type, "roamers", could potentially cover an extensive number of leks with a higher potential possibility to seize copulations.

A mixture of the two types is likely the most realistic scenario and may very well be connected to the age of the males. Youngest birds could be assumed to be "roamers" whereas males close to their prime may start off as "remainers" before the opportunity to establish residence presents itself. A multitude of assessments must be made by a marginal male to remain on a lek. The possibility of locating centrally enough without being evicted by residents and the number and prowess of competitors are at the core of this process. In-between there being too few independents to attract female attention and too many to compete with there is bound to exist a state in the lek where there are too many females for the residents to monopolize, allowing marginals to seize the opportunity (Widemo 1997).
Residents face similar challenges, which they may resolve by setting up a new lek altogether (Hogan-Warburg 1966). Otherwise their actions are dictated by a different set of assessments. The well-established hierarchies between residents likely arise from individual identification, but the extent of this outside of the lekking season and across multiple years is not known. If the site-faithful males continue to recognize each other from season to season, their internal relationships could prove to have a considerable influence over their actions.
Formation of a partnership with a satellite benefits the resident through increased female interest but comes with a number of consequences such as loss of mating opportunities, disruption and distraction by the satellite and potential sperm competition (Shepard 1975; Van Rhijn 1991), giving rise to the "resident's dilemma" (Hugie & Lank 1997) where a resident is forced to accept a satellite due to female preference.

The satellite strategy contains numerous intricate elements. Through trailing flocks of females, satellites may obtain a more precise perception of the overall number of females in a given area, allowing them to accurately gauge the number of female visitations and thus the benefits of remaining or leaving a given lek. Satellites, like females, must estimate the quality of independents on a lek to decipher the optimal partner. Rather than traditional male-male evaluation, a satellite would have to deduce the location of the partner resident in the internal

hierarchy of the lek and it's capability to win against potential competitors. Thus, the best partner is simultaneously the most potential challenger for the satellite as well. This evaluation must be continuous since an overtly aggressive partner, or emergence of a more successful resident, would negate all the benefits for the satellite.

Relationships between satellites have not been studied in-depth, but internal competition for both females and partners is likely. Instances of two satellites partnering with an independent, and even co-operating with each other (D Lank 2019 personal communication April 23), indicate that satellites are capable of remarkable flexibility and adjustment to multiple circumstances.

The possibility of acquiring matings outside of the leks or multiple leks would further explain how the polymorphism is maintained. This would also introduce a new aspect to the satellite strategy as a whole and raise the question of how much time would be dedicated to pursuing extra-lek copulations in contrast to leks and would an "extra-lek seeker" type of satellite have a distinct behavioural pattern and even fitness to those the "lek-attenders". Their considerably large testis indicate a role of sperm competition in their success and copulations acquired outside of the leks could potentially increase their net matings and therefore chance of siring offspring considerably in comparison to exclusively copulating on leks. Since the nature, or existence, of interactions between satellites and females outside of the leks remain without evidence only speculation is possible at the current level of knowledge.

The male morphs of ruffs have been under rigorous scrutiny, but the females and their possible strategies have attained relatively little attention. As studies on ARTs have mainly focused on males and their ostentatious behaviour and physical features, the more discreet competition between females remains much less examined.

Site-fidelity appears to be prominent in ruffs, especially females show a tendency to nest in the area where they hatched (Van Rhijn 2010). Females must go through a process of evaluations to enter a lek and further to remain and copulate on it. The number of independents appears to be the key factor attracting reeves to leks, with the presence of satellites functioning as a further stimulant. Investigating and copulating on multiple leks may allow the females to diversify the genetics of their offspring (Lank *et al.* 2002) and facilitates sperm competition. If females also engage in copulations outside the leks, of which a single case has been documented by Lank & Smith 1987, this could further promote proliferation of satellites and even faeders.

The expression of the genetic inversion remains elusive. Although faeder females are smaller and reproduce later than their counterparts, any further manifestation of the inversion on the females have not been observed. The method through which females carrying the inversion evade copulations with carrier males, therefore preventing inevitable decrease in offspring fitness, is not understood but remains as one of the most essential questions in ruff

reproduction. Genotype could play a part in the role of satellites arousing females, with carrier females being unaffected or even driven away by the features that draw other females to satellites. The case of the faeder is more complicated, with the swift interception method leaving little room for female choice. If females were able to extent control over their sperm storage tubule system it could provide a risk mitigation mechanism.

The intensity of male attendance on a lek has been observed to be essential to their mating success, suggesting that females memorize males from a lek and recognize the most dedicated individuals (Hill 1991). The short visits with little interactions often performed by females may be related to this process of judging the most tenacious males. The nature of female visitations could be expected to change as the season progresses. Early visitations on multiple leks in a given area would grant the females an overview of the numbers of males present at each lek and allow for estimation of their quality. As evaluation of male quality and devotedness continues the visits could be presumed to lengthen until peak receptiveness is reached and accompanied by a quick copulatory visit to the top male, or males. If devotion to a lek is in the epicentre of mate selection it could diminish the attractiveness of the mobile, female-trailing satellites. This criteria could be avoided by the satellites through being specific in the females that they follow, rather than pursuing them sporadically, and thus being constantly in the presence of a female. The extent of female movements before nesting are not well studied, although it would provide a crucial piece of information regarding their tactics and partner choices. Affinity for nesting near leks may indicate that females use each other as units of measure for leks with high quality mates and for nest sites as well. Annual variation of seasons, such as that between 2017 and 2018, may affect both females and males by constraining their range and making it profitable for the birds to settle at other locations than their preferred areas.

8.0 CONCLUSIONS:

8.1 Answering the key questions

The obtained data allowed for investigation of each of the hypotheses presented in section 4.0 (pp 18):

1. Resident male presence was found to have a major influence on the interest and visitation length of females. Marginal males had a more indirect role in augmenting the attractiveness of a lek through increasing the total numbers of males present.

2. Time spent on leks by satellites did not directly attract females, although their presence added to the overall attractiveness of a lek by increasing the numbers. The presence of satellites was found to influence both obtaining and lack of copulations by both independents and satellites alike

3. Satellite presence was found to be influenced by the activity of the females, further indicating that trailing of females rather than dedication to a lek is an essential element in the satellite strategy

4. Similarly to females, the presence of resident males influenced satellite the interest of satellites in a lek. The marginal males played an indirect role through bolstering the size of a lek and thus its attractiveness.

5. Females exhibit a clear peak in both receptiveness and copulations, which however do not completely overlap. The copulation peak is preceded by a period of lek visits that are devoid of interactions until a rapid, synchronized burst of receptiveness by a large number of females.

6. Very few copulations were observed in 2017 but all except the single marginal observation were obtained by residents and satellites on the largest two leks. In 2018 a small number of resident males monopolized the observed matings, with occasional satellite successes. Copulations largely coincided with days of highest lek attendance by all ruffs.

8.2 Review of methodology

The filming method provided large amounts of highly accurate data from the leks. The ability to rewind and slow down the footage to be certain of the movements of individuals and re-watch interesting situations left little room for error. Theoretically the video material gives a perfect picture of all events and numbers of birds visiting a lek, as well as visit durations and peak times of the day. The set-up is easy and relatively cheap.
Unexpected issues can render footage unusable, such as glare from water or the birds moving too far for recognition. The battery life of the camera, even with a power bank attached, limited the amount of filming that could be done in a day.

The video footage from different sites was often from varying dates and times of day and not optimally overlapping. Synchronizing the filming dates and times of day at several locations would yield a precise picture of the local lek system. This would require considerably more resources and efforts from multiple personnel on the field throughout the season. Examining the footage was time consuming. A large portion of the footage is uneventful. Intensive episodes of rapid action could be over in seconds but require several examinations to record arrivals, departures and movements of individuals with certainty. Material from multiple leks for numerous days could in the worst case accumulate into enormous amounts that would require the input of multiple examiners to process.

In addition to the video material, ruffs were meticulously observed outside of the leks but the observations with scopes and binoculars have proved infeasible for obtaining a definitive understanding of their movements. Independent males proved easy to individually recognize and thus monitor on the leks which especially the residents barely left. Marginals were more problematic to identify since only few remained on leks for extended periods and the footage did not allow exact recognition of more subtle features in their plumage. Faeders could not be identified at all, since the only cue would have been a copulation event that was never observed. Catching and colour banding males has proven challenging in the past and was not attempted on either year, although devising a method to reliably catch multiple males without causing potential abandonment of the lekking site would give invaluable information on male movements when identities of marginals could be verified. There have been undertakings of male colour banding and attempts to devise more efficient methods (K Koivula 2017, personal communications, May 15). Colour banding females has allowed individual recognition, which in the case of reeves would otherwise be extremely difficult if not impossible. The numbers of observed banded females have risen annually, allowing for more information the be gathered each year.

A major step in understanding not just female movements, but also the possible variation in post-lekking behaviour of males according to status and morph, would be precise tracking of the birds through radio- and GPS-tags (Verkuil *et al.* 2010; Scarpignato *et al.* 2016). This would allow deciphering key features such as the number of visited leks by both males and females, with the potential of discovering new sites, and how large of an area they monitor before settling to nest.

8.3 Guidelines future studies

The results presented in this thesis have provided further evidence of the complexity of the relationships that control the reproductive cycle of the ruff and suggest that many aspects of it remain unexplored.

The bilateral influence that the satellite males and females exert on each other appears as one of the most intriguing features of the ruff lek, especially since satellite presence appears to also affect the copulation rates of the resident independents. The connections between the two could be investigated through precise observation of both groups throughout the breeding season. If satellites assess female visit rates, their visit rates and length of attendance during the latter portion of the season could be explained by the quantity of female visits over the earlier part of the season. If instead it is the females that appraise satellite attendance the reverse would occur. The details of the satellite strategy in general include multiple unknown factors, such as their activity patterns during the breeding season and how their movements are altered depending on lek constitution, female movements and progress of their receptiveness. If pursuit of females is a key factor during peak receptivity it should be discernible from observations of arrivals and embarkments of females from leks and if satellite actions correspond with them. On the contrary resident males should display their highest degree of dedication to their lek at this point.

The considerable time and effort investment in male appraisal by females indicated by the low copulation rates per visit, as well as the possibility of different female tactics perhaps linked to age and morph are topics through which the far less studied female perspective of ARTs could be investigated.

The lekking and nesting seasons give only a limited amount of information of the full life cycle of the ruff. Field work directed at the wintering grounds could increase understanding of function of social hierarchies and possible unknown selective pressures operating outside of the breeding season.

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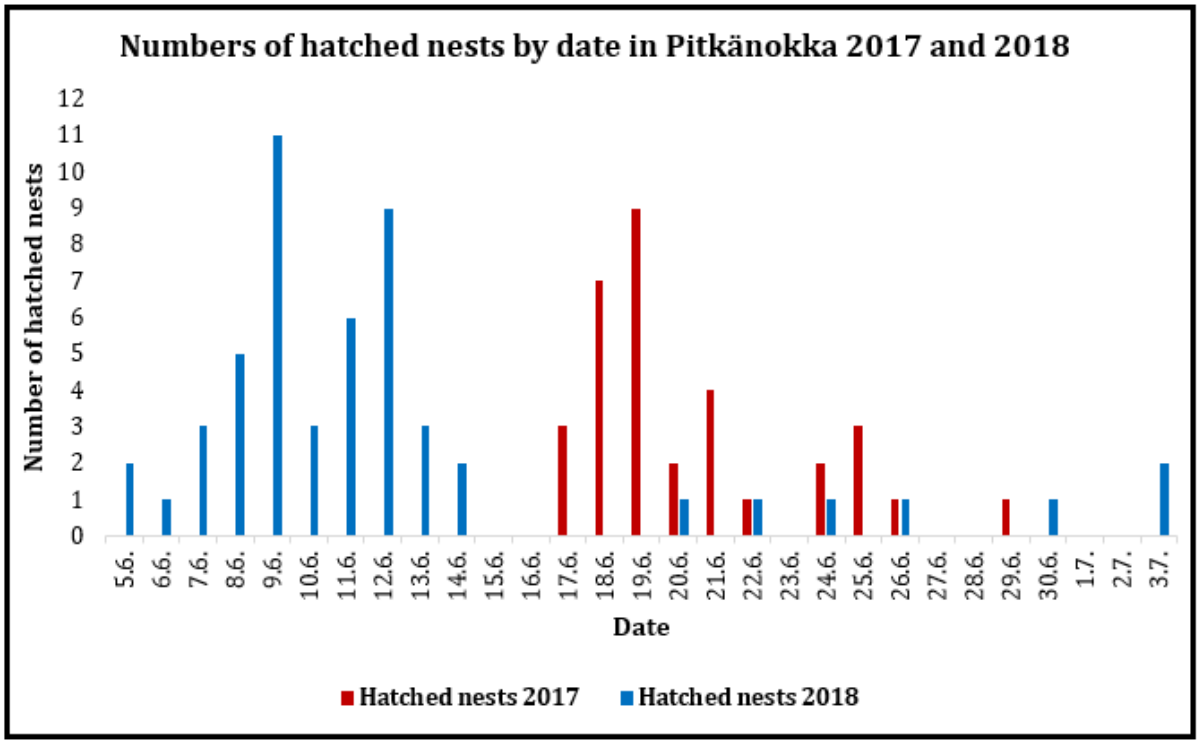
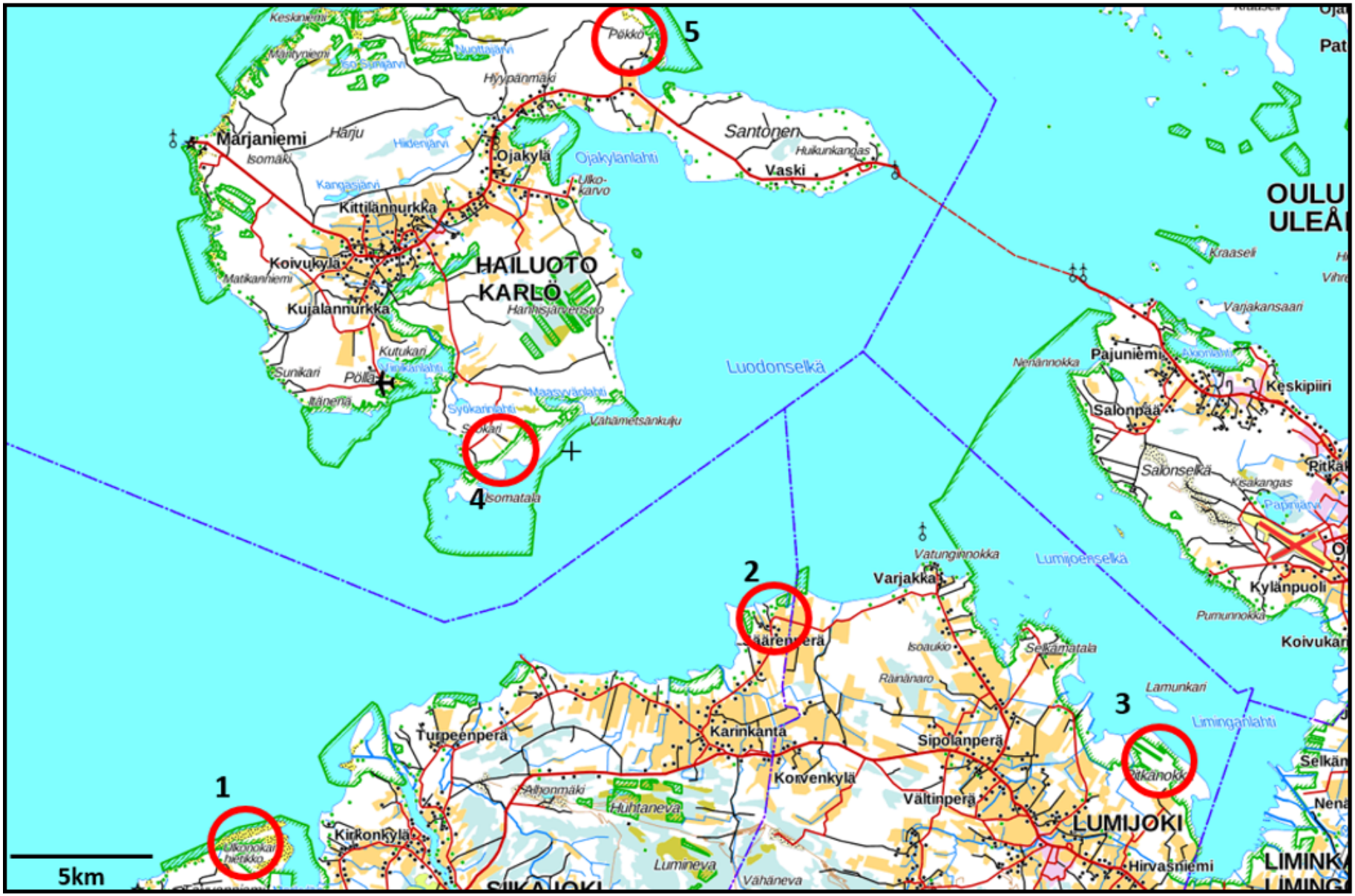
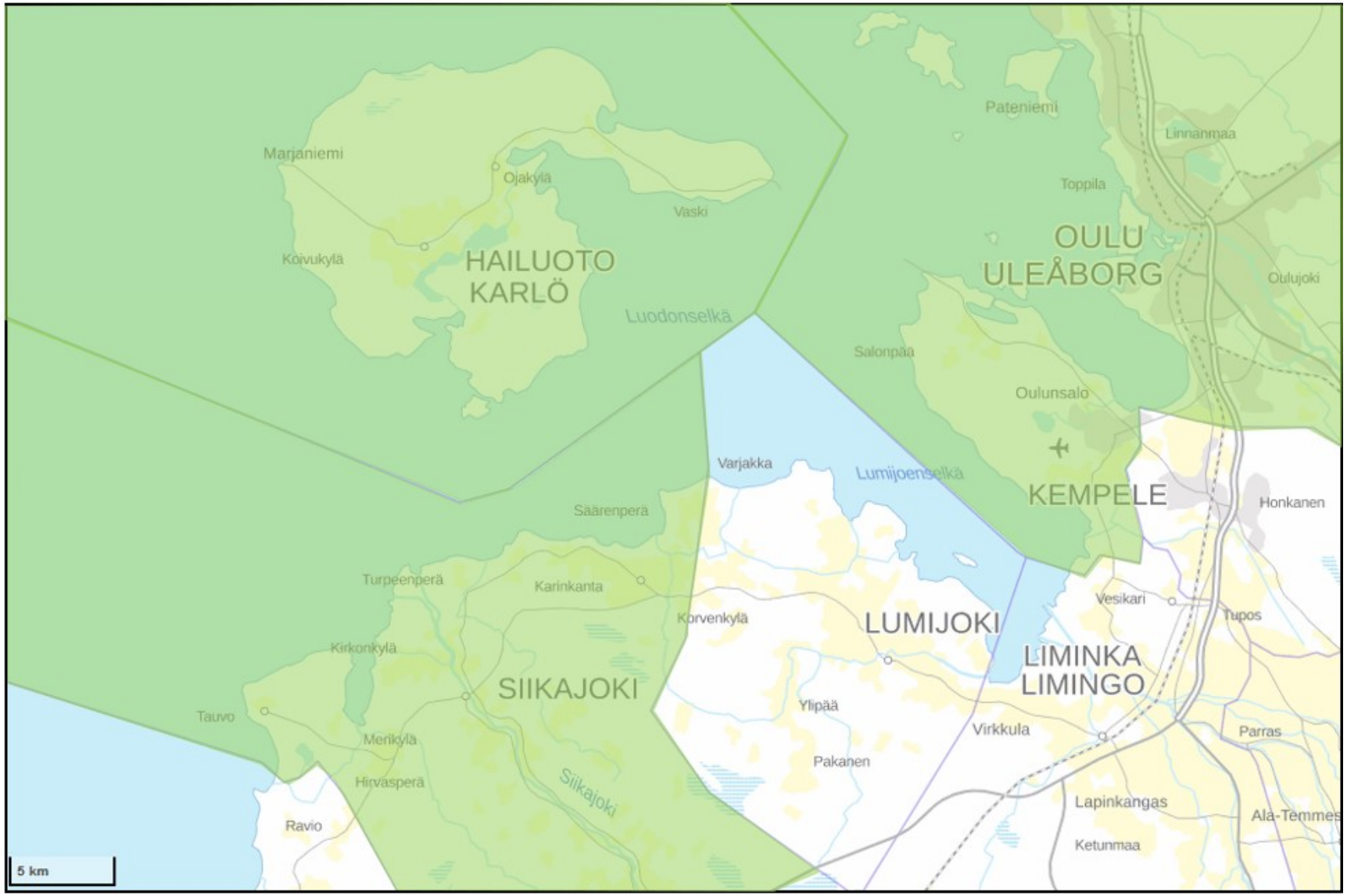
Supplementary materials



Appendix 1. Number of hatched nests in Pitkänokka 2017 and 2018



Appendix 2. Map of the field sites with coordinates in ETRS-TM35FIN. **1.)** Tauvo, *N 7191183,838, E 387819,089* **2.)** Säärenperä *N 7199207,068, E 403841,858* **3.)** Pitkänokka, *N 7194267,833, E 417840,054* **4.)** Tömppä *N 7204909,500, E 396358,840* **5.)** Pökkö *N 7217961,576, E 401054,567.* Source: Maanmittauslaitos / National Land Survey of Finland (2019).



Appendix 3. Geographical area from where the observational ruff data from Birdlife Finland's Tiira database (Birdlife Finland 2019) was collected (in green). Source: Maanmittauslaitos / National Land Survey of Finland (2019).